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Acute and Chronic Parathion Toxicity to Fish and Invertebrates

Union Carbide Corp.
Tarrytown, NY

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ACUTE AND CHRONIC PARATHION TOXICITY TO FISH AND INVERTEBRATES

by

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Union Carbide Corporation Environmental Services

Contract No. 68-01-0155

Project Officer

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Acute and chronic aquatic bioassays were conducted with a variety of organisms using parathion [0,0-diethyl 0-(p-nitrophenyl) phosphorothioate] as the challenge compound. Acute LC50 values ranged from a low of 0.38 µg/l in invertebrates to a high of 2.0 mg/l in freshwater fish. Non-lethal effects were documented in bluegill and brook trout chronically exposed to parathion concentrations in excess of 0.17 µg/l and 32.0 µg/l respectively. Chronic exposure of fathead minnows to concentrations of 4 µg/l resulted in deformation and reproductive impairment. Bioconcentration of parathion in freshwater fish tissue ranged from five to several hundred times that of the exposure water. Chronic no effect concentration of parathion for D. magna was 0.08 µg/l, for G. fasciatus less than 0.04 µg/l and for C. tentans less than 3.1 µg/l.

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FOREWORD

This report constitutes the last in a series describing research projects intended to: 1) generate additional freshwater aquatic life toxicity data useful for evaluating environmental hazards of commonly used pesticides; 2) advance the state-of-the-art of chronically testing the species involved; 3) evaluate the ability of relatively inexperienced investigators to successfully accomplish chronic studies with these species and thus the advisability of recommending them for routine tests through such documents as the Pesticide Registration Guidelines; and 4) provide data required to evaluate the validity of the application factor hypothesis. These studies have been useful in the above regards and have therefore contributed importantly to both basic and applied toxicology.

John G. Eaton, Project Officer
Environmental Research Laboratory-Duluth

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ABSTRACT

Acute and chronic aquatic bioassays were conducted with a variety of aquatic organisms using parathion [O,O-diethyl O-(p-nitrophenyl) phosphorothioate] as the challenge compound. The bioassay organisms challenged in this study and their resultant 96 hour LC50 values were: bluegill sunfish, Lepomis macrochirus Rafinesque, 0.51 mg/l; brook trout, Salvelinus fontinalis (Mitchill), 2.00 mg/l; fathead minnow, Pimephales promelas Rafinesque, 1.6 to 0.5 mg/l; water flea, Daphnia magna Straus, 0.63 ug/l; scud, Gammarus fasciatus Say, 0.4 ug/l; and midge, Chironomus tentans (Fabricius), 31.0 ug/l. Non-lethal effects such as deformities and acetylcholinesterase depression were documented in bluegill chronically exposed for 23 months to parathion concentrations in excess of 0.17 ug/l. Adult brook trout exhibited no toxic symptoms at 7.2 ug/l parathion exposure but egg hatchability was reduced at an exposure concentration of 32 ug/l. Chronic exposure of the fathead minnow to a parathion concentration of 4 ug/l for 8-1/2 months resulted in adult deformities and decreased egg production. Parathion residues detected in exposed bluegills were 5 to 25 times that of the exposure water while trout exhibited a bioconcentration of several hundred times that of exposure water. Parathion residues in the fathead minnow were detected as intermediate between bluegill and trout. Juvenile and adult stages of chronically exposed fish appeared to be the most sensitive to parathion. The chronic no-ill-effect concentration for D. magna was 0.08 ug/l, for G. fasciatus it was less than 0.04 ug/l and for C. tentans it was less than 3.1 ug/l. Mean LC50 concentrations for D. magna (48 hour) and G. fasciatus (96 hour) were 1.13 ug/l (n = 2) and 0.39 ug/l (n = 4) respectively.

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SECTION I

INTRODUCTION

Among organophosphorus insecticides, parathion is one of the most persistent and toxic. About 15 million pounds of it are used annually for control of insect pests on fruit trees, cotton, tobacco, and other crops (1). A small portion reaches aquatic environments by direct application (such as for mosquito control), land runoff, leaching, erosion, irrigation, aerial spraying and spills during manufacturing, formulation, and application. Once in a water-soil system, its rate of degradation is variable and dependent on pH, organic control, and microbial activity. Upon application to soil, parathion may persist for three months (2), five years (3), or in an extreme case, 16 years (4). In distilled water of pH 7.4, the hydrolysis reaction has a half life of about 108 days, decreasing with increasing pH and temperature (5). In contrast, natural river water degrades 50 percent of parathion within a week (6). In natural lake sediments Graetz et al (7) found a half life of 178 to 68 days.

Damage to aquatic fauna has been reported for parathion residues in water and sediments. After spraying an orchard, Nicholson (8) found reduced populations of aquatic insects in nearby ponds containing 1.9 ppm in mud. Killifish and freshwater mussels exposed on a treated cranberry bog showed uptake of parathion residues (9). Parathion spills have also caused kills of fish and amphibians. (10).

Like all organophosphates, parathion disrupts the central and peripheral nervous systems of animals when enzymatically converted to its oxygen analog, paraoxon. Paraoxon then combines covalently with acetylcholinesterase (AChE) to block the hydrolysis of acetylcholine, the transmitter substance in the synapses of motor and parasympathetic nerves. A full account of this inhibition is given by O'Brien (11). The AChE-phosphate complex is, at first, hydrolyzable. Animals may recover from acute doses of the inhibitor. However, the AChE-complex can gradually dealkylate, forming the phosphate anion which resists hydrolysis. Therefore, regeneration of AChE after long-term exposure of parathion is extremely slow.

Weiss demonstrated AChE activity in fish (12), and its susceptibility to paraoxon inhibition (13). Bluegills exposed to 0.1 mg/l parathion for eight hours recovered only 50 percent of normal activity in 30 days. Fathead minnows had a somewhat quicker response and recovery. Carter (14) found that channel catfish with 50 percent inhibition from methylparathion required 20 to 30 days to regain 80 percent of normal activity. Studies of AChE inhibition in vitro have found fish (15, 16, 17) and lobsters (18) to be less sensitive than birds or mammals. Yet parathion is very toxic to both fish and crustaceans. The two principal metabolite products in rats

(19), paraoxon and diethyl hydrogen phosphorothioate, have also been found in rainbow trout (16). The proportion of the two metabolites formed, and their rates of formation, vary with species. Metabolism can be slow enough to permit accumulation of parathion in tissues, as reported for trout (16), brown bullheads (20), mosquitofish (21), fathead minnows (22) and oysters (23).

Literature values for the acute toxicity of parathion to aquatic species have been summarized by Kempt, Abrams and Overbeck (24). Only a few studies have considered long-term effects. Mount and Boyle (20) found convulsions in bullheads exposed to 0.03 mg/l parathion for one month. Matsue (25) observed retarded growth of goldfish at 0.1 mg/l for one month. Reduced growth rates of oyster larvae in 0.05 mg/l were found by Davis and Hidu (26). After 40 days at 0.01 mg/l, male guppies tested by Billard (27) showed reduced spermatogenesis. Jensen and Gaufin (28) reported a 309-day LC50 of 2.2 ug/l for Pteronarcys californica and 0.44 ug/l for Acroneuria pacifica.

Past research demonstrates that parathion is highly toxic to aquatic species, may accumulate in tissues, and may persist long enough to cause chronic damage. To find ecologically "safe" levels allowable in aquatic systems, the long-term effects of parathion must be studied further. The objective of the present study was to find the highest chronic concentration of parathion that produced no harmful effects to brook trout, bluegills, fathead minnows, Daphnia magna, Gammarus fasciatus and Chironomus tentans. For each species, the maximum acceptable toxicant concentration (MATC), defined by Mount and Stephan (29), was then related to the acute LC50. Because standardized bioassay techniques were used, the results of these tests may be correlated with those for other species and compounds. The combined information will facilitate the prediction of chronic no-effect levels in fresh water.

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SECTION II

CONCLUSIONS

1. Juvenile bluegills were found to have a 96-hour median lethal parathion concentration (LC50) of 0.51 mg/1. A maximum acceptable toxicant concentration (MATC) between 0.17 and 0.34 ug/1 was established based on adult deformities.

2. Brook trout had a 96-hour LC50 of 2.00 mg/1. Chronic exposure to a concentration of 7.2 ug/1 produced no harmful effects. However, levels of 32 ug/1 reduced hatchability of embryos from previously unexposed parents.

3. Fathead minnows exhibited 96-hour LC50 values between 1.6 and 0.5 mg/1 and a MATC of approximately 4 ug/1 based on egg production data and incidence of adult deformities.

4. Daphnia magna exhibited a mean 48-hour LC50 of 1.13 ug/1 and a MATC of approximately 0.08 ug/1.

5. Gammarus fasciatus had a mean 96-hour LC50 of 0.39 ug/1. The MATC was determined to be lower than 0.04 ug/1, a concentration which caused mortalities in one month.

6. Chironomus tentans had a 96-hour LC50 of 31 ug/1 based on a measured parathion concentration in water. The MATC was determined to be less than 3.1 ug/1, a concentration which produced lethality in two weeks.

7. Under chronic conditions bluegill tissue concentrated parathion residues at 5 to 25 times the exposed water concentration. Brook trout concentrated parathion several hundred times and minnows at rates intermediate to the other two species. No parathion above background levels was detected in D. magna.

8. Juvenile and adult developmental stages of chronically exposed fish appeared relatively more sensitive to parathion induced effects than egg and fry stages.

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SECTION III

RECOMMENDATIONS

1. The concentration of parathion applied to bodies of water should be limited to levels acutely tolerated by crustaceans such as Daphnia.

2. Trout and other fish species collected from parathion-treated sites should be examined further for the presence of potentially harmful parathion residue levels.

3. Other insecticides should be evaluated as possible alternatives to parathion in regard to their selectivity, toxicity and persistence.

4. Food chain dynamics of parathion distribution in aquatic communities should be investigated.

5. Metabolic pathway studies of parathion degradation in fish should integrate the effects of temperature, rates of reaction, and proportion of major metabolites.

6. Further histological characterization of parathion damaged fish tissues would help to determine the mechanisms of such damage.

7. Further culture information is needed to ensure bluegill spawning during chronic tests if such studies are to be continued with this species.

8. Five month chronic tests using Gammarus as the bioassay organism are not practical unless better survival rates can be achieved in culture situations.

9. More efficient techniques are needed for conducting the egg incubation and larval survival phases of chronic bioassays.

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SECTION IV

MATERIALS AND METHODS

WATER SUPPLY AND ENVIRONMENTAL CONTROL

Water Supply

Water used for conducting the acute and chronic tests of the present study was supplied from a 50-foot well located on the Tarrytown, New York, laboratory site. Results of a chemical analysis of ambient temperature well water according to the methodology of Standard Methods (30) are given in Table 1. From the well, water was pumped to the testing laboratory through cast iron and PVC pipe. At the laboratory the water was sterilized with ultraviolet light and proportioned into lines of ambient (11-13°) and heated water for distribution to each of the testing stations. Heated water utilized for conducting relatively warm water tests was softened by exchange of sodium for calcium. This "softening procedure" consequently resulted in a 20 percent hardness reduction in the bluegill chronic exposure.

TABLE 1. ANALYSIS OF TEST WATER

Component	Concentration, mg/l	Component	Concentration, mg/l
Alkalinity (as $CaCO_3$)	147	Lead	<0.0002
Aluminum	0.01	Magnesium	27
Ammonia (as N)	0.15	Manganese	0.005
Arsenic	<0.017	Mercury	<0.0002
Barium	0.10	Nitrate (as N)	1.7
Boron	0.02	Nitrite (as N)	0.007
Cadmium	0.005	Phenol	<0.0016
Calcium	76	Phosphorus	0.005
Chloride	130	Potassium	0.6
Chlorine	<0.001	Silicon	6
Chromium	<0.006	Sodium	3.9
Copper	0.03	Sulfate	39
Cyanide	<0.0001	Strontium	0.6
Fluoride	0.02	Tin	0.01
Hardness (as $CaCO_3$)	297	Titanium	0.01
Iron	<0.0006	Zinc	<0.001

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Temperature Control

Separate lines of both hot and cold water supplied each test system. Proportional mixing of the two thermal flows was regulated by a pneumatic temperature controller with a sensing element in the mixing zone of the test system supply pipe. The resulting test water temperature variation was within ±1°C for instantaneous changes, and ±2°C for daily trends. The ambient temperature in at least one exposure chamber per test system was continuously monitored by a thermistor probe read by a 20-channel electronic temperature monitor. All channels monitoring test systems had individual high and low temperature set point designations which activated an alarm if exceeded. Each hour, the temperatures at all channels were recorded on punch paper tape and teletype.

Aeration

To diminish the possibility of well water supersaturated with gases from entering the test systems and inducing variable mortality, water was aerated by air-stone as it entered the first proportional diluter cell. In addition, adult fish exposure chambers had supplemental aeration to ensure dissolved oxygen concentrations greater than 60 percent saturation.

Toxicant Delivery

Reagent grade (99 percent pure) parathion [O,O-diethyl O-(p-nitrophenyl) phosphorothioate] obtained from Pfaltz and Bauer was used in the preparation of all test stock solutions. Stock solutions of parathion in either water or acetone were injected into proportional diluters of the Mount and Brungs design (31). Depending on test type, various injection devices were used: Sage 341 syringe pump, a Mariotte bottle with constant flow, mechanical syringe pump (31), or LKB Perpex peristaltic pump. To prevent accidental injections during diluter malfunctions, the electrical pumps were wired to operate only during normal diluter cycles.

All components of the diluter in contact with exposure water were either of glass, latex or Tygon tubing and silicone adhesive. The diluter produced five concentrations of parathion, plus a control, each split into individual duplicate flows by a mixing cell. Tanks were designated by the same number as the corresponding diluter cell (31): "0" for the control, "1" for the highest toxicant concentration used, "2" for the second highest, up to "5" for the lowest. Duplicate tanks were individually identified by "A" or "B" designations.

Exposure tank sizes and flow rates for each of the chronic test systems are summarized in Table 2. The bluegill adult exposure tanks were constructed of No. 306 stainless steel. Trout, minnow and Gammarus adult exposure tanks were commercial glass aquaria with slate bottoms and outside stainless steel reinforcement. All other test containers were of glass and silicone adhesive (Dow Corning) construction.

TABLE 2. SUMMARY OF CHRONIC TEST CONDITIONS

Test animal	Adult tank volume, liters	Minimum flow per tank, liters/hour	Larval chamber volume, liters	Temperature deg C
Bluegill	170	40	6*	25
Brook trout	85	30	6*	9-15
Fathead minnow	28	10	7*	28
Daphnia	3	0.8	...	18
Gammarus	15	0.5	...	18
Chironomus	3	0.8	...	25

*Two such chambers were used per adult tank. Minnow chambers were not true duplicates. See page 12.

Lighting

All tests were illuminated by Duro-Test Optima fluorescent lamps. These were regulated by Tork industrial timers to provide appropriate Evansville, Indiana, photoperiods as shown in Table 3. The timer wiring was modified to permit 30 minutes of gradual photointensity change at dawn and dusk (32). All Daphnia and midge tests were conducted under a 16-hour light, 8-hour dark photoperiod. Average light intensities at the test exposure systems were: bluegill, 75 ft-c; brook trout, 70 ft-c; minnow, 82 ft-c; and invertebrates, 70-80 ft-c. While photoperiods for bluegill and brook trout exposures corresponded to actual calendar dates, the minnow and invertebrate tests were begun on the Evansville, Indiana, dates shown in Table 3.

FISH BIOASSAYS

Bluegill Sunfish (Lepomis macrochirus) Parathion Acute and Chronic Exposures

Bluegill sunfish (Lepomis macrochirus) used in the parathion chronic bioassay were collected as 5-8 cm long (total length) juveniles from a naturally occurring pond population located in Dutchess County, New York. Prior to their use in the chronic exposure, these bluegills were acclimated to laboratory conditions at the Union Carbide facilities in Tarrytown, New York, for a period of three months. A second stock of immature bluegill sunfish, taken from the same pond one year later, were isolated and acclimated at the Union Carbide facilities and used for the flow-through acute studies. All bluegills selected for testing were relatively free of internal parasites but initially harbored gill ectoparasites which were subsequently eradicated during the acclimation periods by therapeutic formalin treatments.

Bluegill parathion acute bioassays consisted of five (four preliminary and one definitive) tests conducted at 22°C under flow-through conditions. The acute definitive bioassay was performed using five challenge concentrations of parathion plus a control in duplicate over a 144-hour exposure period in 85-liter glass aquaria.

TABLE 3. CHRONIC TEST PHOTOPERIOD AND SCHEDULE

Date	Light period, hours:minutes*	Event
Jan 1	10:30	
15	10:45	
Feb 1	11:15	Begin *Gammarus* test
15	11:45	
Mar 1	12:15	Begin brook trout test
15	13:00	
Apr 1	13:30	
15	14:15	*Gammarus* reproduction
May 1	14:45	
15	15:15	Fathead minnow spawning
June 1	15:30	
15	15:45	
July 1	15:45	Bluegill spawning
15	15:30	
Aug 1	15:00	
15	14:30	
Sept 1	14:00	
15	13:30	
Oct 1	12:45	Begin bluegill test
15	12:15	
Nov 1	11:30	
15	11:00	Brook trout spawning
Dec 1	10:45	Begin fathead test
15	10:30	

*Includes 30 minutes of dawn and 30 minutes of dusk.

Parathion stock solutions for the flow-through acute tests were prepared as aqueous sonicated emulsions consisting of 180 gm parathion and 8 gm Triton X-100^{R} (Rohm and Haas) per liter of water. Five challenge concentrations were prepared by volumetrically dispensing appropriate amounts of parathion stock solution via peristaltic pump into a proportional diluter which provided a flow of 30 liters per hour to each of the 85-liter test aquaria.

Direct analyses of the parathion challenge concentrations in each of the definitive test exposure aquaria were made at test initiation and at 48 hours. The definitive acute bioassay was considered started when stock bluegills, acclimated to test conditions without feeding, were randomly distributed to each of the duplicate challenge concentrations at a rate of five per aquarium. At regular time intervals, during the 144-hour exposure period, each of the exposure aquaria was examined for bluegill mortalities or signs of toxicant related stress. At no time during the definitive test were fish fed or was aeration provided to exposure aquaria.

The parathion bluegill sunfish chronic bioassay was conducted following procedures from the "Recommended Bioassay Procedure for Bluegill Sunfish *Lepomis macrochirus* (Rafinesque) Partial Chronic Tests" of the National Water Quality Laboratory, Duluth, Minnesota (revised January, 1972). A flow rate

of 40 liters per hour was accomplished in each of the exposure tanks by use of a Mount and Brungs type proportional diluter calibrated to provide nominal parathion challenge concentrations of 8.0, 4.0, 2.0, 1.0 and 0.5 ug/l. Stock parathion was injected by syringe pump to the diluter apparatus as a solution of 2 to 4 gm parathion and 0.24 gm Triton X-100 per liter of acetone.

The parathion bluegill chronic exposure commenced on December 22, 1971, with the random distribution of 20 bluegills into each of the exposure tanks. The photoperiod was adjusted at the start to correspond to that of Evansville, Indiana, in October (Table 3). All fish were fed Oregon Moist Pellets and Silver Cup trout food ad libitum and had their lengths and weights recorded at the beginning of testing, at thinning, and at the test's termination.

During the first two months of exposure, bluegills exposed to the 8.0 ug/l exposure concentration experienced tremors and convulsions. This necessitated termination of the exposure series after 64 days and a new 0.25 ug/l exposure concentration was added to the chronic as a replacement.

Development of normal male territorial aggression and spawning behavior during testing did not occur until the second summer. At that time all exposure tanks had their bluegill populations thinned to 3 males and 7 females. Surplus fish from each exposure concentration were frozen at -18°C for subsequent chemical analyses. To simulate spawning beds, a cement substrate, 27 cm square by 5 cm thick with a central shallow depression, was placed at the end of each exposure tank. Two black plastic curtains (55 x 40 cm) serving as visual barriers were also partially submerged wihin each exposure tank to diminish aggression between spawning males.

During the spawning period, all substrates were checked daily for eggs. Eggs from all individual spawns were collected and counted by volumetrically enumerating the number of eggs in one milliliter and multiplying by the total volume of eggs spawned. Two hundred eggs from each spawn were incubated in egg cups constructed from bottomless 4-oz glass jars with 40 mesh nylon screen glued to the open bottoms. All egg cups were suspended by an oscillating rocker arm assembly in larval chambers corresponding to the exposure tank in which the incubating eggs were spawned. The 6-liter larval chambers were 13 cm wide, 35 cm long and 18 cm high and contained a 13 cm depth of exposure water which was received directly from their corresponding adult exposure tanks via a continuous siphon system. After a three-day incubation the hatched fry were counted and their numbers recorded and 50 live fry were retained suspended in the larval chambers. The fry were retained for seven days in the cups and fed newly hatched Artemia salina four times daily. At seven days post hatch, fry were released into the larval chambers and mortalities were recorded weekly. Surviving fry in each exposure larval chamber were counted and measured at 30 and 60 days post hatch. Measurements were done by placing surviving fry from each larval exposure chamber in an all glass chamber set over a light box having a millimeter transparent grid and taking a photograph relative to the grid image. The test was terminated after 23 months of parathion exposure and all remaining adult bluegills were examined and frozen for residue analysis.

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Brook Trout

Yearling brook trout, Salvelinus fontinalis (Mitchill), for use in the parathion acute and chronic exposures were obtained from Sullivan County, New York. The trout, approximately 60 gm each in weight, were acclimated at 12°C for three weeks prior to chronic testing. Trout used in both the acute and chronic tests showed no disease symptoms prior to testing. During chronic testing, therapeutic treatment for fungus and furunculosis was accomplished using 20 ppm formalin, 4 ppm Furpyrincl or 40 ppm malachite green dips. Two preliminary and one definitive flow-through acute brook trout bioassays were conducted following the bluegill acute bioassay methodology but at 12°C. Toxicant was supplied to the acute exposure tanks as an aqueous emulsion of 0.05 gm Triton X-100 per gram of parathion. This was injected into the diluter by peristaltic pump. Water for parathion analysis was sampled at the initiation of testing and at 2, 4 and 5 days of test exposure. Selected fish were frozen at -18°C for later residue analysis.

The flow-through parathion chronic brook trout exposure followed the procedure given by the National Water Quality Laboratory (34). On May 1, 1972, fourteen fish were randomly distributed to each of the 12 exposure tanks. A stock solution of parathion was prepared using 8 gm of parathion and 0.24 gm Triton X-100 per liter of acetone and was dispensed into the chronic diluter system by syringe pump. The chronic physical system was identical to that used in the flow-through acute exposures (flow of 30 liters per hour in 85-liter aquaria) and provided a maximum theoretical acetone concentration of 1.5 mg/l in the highest exposure concentration series used.

Trout in the chronic exposure were fed Silver Cup trout food according to the manufacturer's recommended daily ration. Weights and total lengths of chronically challenged trout were recorded at the start of testing, after 117 days exposure, at 178 days exposure (thinning, discards only) and after 282 days exposure (termination). Trout in the chronic exposure were thinned to two males and four females per tank at 178 days in anticipation of spawning. Secondary sexual characteristics made it possible to distinguish the sexes of most, but not all, of the fish. Surplus trout were dissected and the tissues frozen for residue analysis.

After thinning of trout from the chronic system (day 206 of exposure, November 22, 1971), two polyethylene basins, 25 cm wide, 30 cm long and 15 cm deep, were placed in each tank as artificial spawning substrates. A horizontal layer of stainless steel screening with small rocks glued to the surface was then placed about 5 cm from the bottom of the substrate. Work by Benoit (35) indicates that this type of substrate necessitated a system of baffles to prevent eggs from experiencing incomplete fertilization.

Eggs were removed from the substrate each afternoon, and counted manually. Fifty eggs from each spawn were incubated in egg cups suspended in shaded larval chambers. The larval chambers in both chronics received exposure water at a rate of 2.6 liters per hour directly from the mixing cells of the diluter. Additional eggs from each spawn were incubated for 12 days and examined for viability. Under low magnification the embryonic develop-

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ment could be seen when the eggs were preserved in an aqueous solution of 5 percent formalin, 4 percent glacidal acid, and 6 percent glycerin (v/v). Viability was calculated as the percent of embryos with a neural keel. During incubation, the eggs were checked daily for mortalities and fungus. To supplement the hatchability data from the chronic test, additional brook trout embryos from the Nashua National Fish Hatchery, Nashua, N.H., were exposed to parathion levels of 10, 32 and 100 ug/l with a control at 10°C. Toxicant solutions of these concentrations were delivered at rates of 200 ml/hr to each of four larval chambers by constant drip Mariotte bottles. Each chamber incubated two egg cups containing 100 nineteen-day-old embryos each. These were held until hatch. Every other day, the embryos were treated with a 4 mg/l solution of malachite green for one hour to prevent fungus.

On exposure day 282, all but two of the surviving adult brook trout from each exposure aquarium, of the parathion chronic exposure were sacrificed. The sacrificed fish were immediately frozen at -4°C to -18°C while the live fish were held in tanks of flowing clean water from two to seven days. Tissue samples taken from fish held in the flowing water conditions were analyzed for parathion residues.

Fathead Minnows

Fathead minnows, Pimephales promelas Rafinesque, used for bioassay tests were obtained from the Newtown Fish Toxicology Station, Cincinnati, Ohio, and cultured for two years at the Aquatic Environmental Sciences laboratory. A preliminary static acute test and a definitive flow-through acute using adult fathead minnows were conducted at 22°C and 24°C respectively. The definitive flow-through acute test was conducted by the same procedure used for the bluegill acute bioassays. Parathion stock was made as an aqueous sonicated emulsion of 29.0 gm parathion and 1.45 gm Triton X-100 per liter of water and injected into the diluter apparatus by peristaltic pump. The flow rate to each 19-liter test tank was approximately 15 liters per hour in all acute tests.

The chronic exposure followed the procedure of the National Water Quality Laboratory (34). Thirty-five minnow larvae, 5 to 14 days old, were randomly distributed to each of the exposure tanks on April 1, 1972 (Evansville date, December 1). Minnow fry were fed a mixture of powdered fish food, mixed with green algae, and Artemia nauplii for the first 30 days. After 30 days minnows were fed a commercial trout starter mash ad libitum each day.

The nominal parathion exposure concentrations selected for testing were 50, 25, 12, 6 and 3 ug/l. These exposure concentrations were produced from a stock solution of 1.7 gm parathion and 0.1 gm Triton X-100 per liter acetone injected into a proportional diluter by peristaltic pump.

At 30 and 60 days exposure, minnow fry from each replicate exposure concentration tank were counted and photographically measured (33). At 70 days exposure the minnows in each tank were thinned to 15 fish and three artificial spawning substrates were added. Substrates were made from 4-inch sections of 3-inch diameter asbestos and cement drain pipe, bisected longi-

tudinally.

Spawning began on October 11, 1972 (June 22, Evansville test date) and continued through December 19 (August 19, Evansville test date) at which time the test was terminated. During the spawning period, substrates were checked daily for eggs. When spawns were encountered, eggs were counted and fifty eggs from each spawn were incubated in an egg cup suspended in a larval chamber receiving water from the corresponding parent tank. Each larval chamber was 30 cm square by 15 cm deep, divided into two sections. Although these two sections physically separated distinct groups of spawned fry, they were not true duplicates since each received the same water.

Prior to incubation, all eggs were treated once with a solution of 4 mg/l malachite green to prevent fungal infection. Larvae were counted after hatching was completed, usually in five days. Of those hatched, 40 were released into the larval chamber for 30-day survival tests. Because a large excess of eggs was produced in the controls, some of these were also hatched at high parathion concentrations. At 30 days all larvae were counted and measured photographically (33). After 8 months of parathion exposure, the adult minnows were sacrificed, examined for deformities, measured, and frozen at -18°C for residue analysis.

INVERTEBRATE BIOASSAYS

Daphnia magna

A stock of Daphnia magna Straus originally obtained from the National Water Quality Laboratory, Duluth, Minnesota, was continuously cultured throughout the project in 3-liter jars which had become coated with diatoms and other algae. Cultures were maintained under static conditions at temperatures of 18 to 21°C, conditions which proved adequate for reproduction. A stock food supply of 1.5 gm Cerophyl, 2.0 gm trout mash, and 1.0 gm yeast in 500 ml well water was prepared and a few ml of this mixture were added periodically to each culture tank. Cultured daphnids were transferred to clean culture jars monthly as a precaution against excess waste accumulation.

In preparation of acute or chronic bioassays, several dozen gravid females were selected from culture and held in clean 250-ml beakers overnight. Within a 24-hour period enough first instar daphnids were usually produced to initiate testing. Instars were counted and randomly distributed to each of the exposure test vessels. In an acute bioassay the number of surviving Daphnia were counted daily and in chronic studies survivors were counted weekly. Progeny produced during chronic tests were counted and discarded each week. Size differences between adults and progeny enabled these two life stages to be distinguished. Daphnia in all tests were fed a daily ration of 5 ml per test container of the food mixture.

All Daphnia bioassays were conducted using 3-liter glass jars as the exposure vessels. Each exposure vessel had a U-shaped notch cut into the upper lip which was covered with No. 405 Nytex screen. This configuration permitted establishing a constant water volume in the test vessels without loss of test organisms. Exposure temperature was maintained constant by

partially submerging all exposure vessels in a constant 18°C water bath. A flow of exposure water at the rate of 0.8 liter per hour was provided to all test vessels via a proportional diluter.

Gammarus fasciatus

Adult and immature Gammarus of several closely related species were collected in coves and tributaries of the Hudson River in an area above the salt wedge. Collection methods included wire cage traps weighted with rocks and dip nets. Subsamples of the population used for testing were identified as G. fasciatus by Dr. E. L. Bousfield of the National Museum of Canada, Ottawa, Ontario, and by Dr. S. Ristich of the Boyce Thompson Institute, Yonkers, New York. Because immature and female gammarids are extremely difficult to identify, the possibility cannot be excluded that some other species were included in the test population of G. fasciatus.

Tests were started with juvenile gammarids obtained from field collections because laboratory cultures did not produce sufficient young.

All tests (acute and chronic) were performed in 15-liter aquaria maintained at 18 to 20°C. Each exposure tank was supplied with several square centimeters of well-soaked maple leaves for cover and food. During the chronic exposure tests and in culture, Gammarus were fed small cubes of beef liver daily. This food source gave higher survival rates than dried fish food, aquatic macrophytes, or maple leaves alone.

Bioassays were conducted using a proportional diluter supplied with a 200 mg/l stock solution of parathion in acetone. The maximum possible acetone concentration achieved at this stock mix was about 7.5 mg/l in both the acute and chronic tests.

Although a full 5-month chronic exposure starting with newly hatched gammarids was originally planned, it was started five times but each time encountered difficulties in organism survival or parathion level. However, two chronic exposures of juvenile gammarids for 35 and 43 days were completed and estimates made of the LC50 levels for these exposure periods.

Chironomus tentans

A stock of Chironomus tentans Fabricius provided by Dr. William Cooper of Michigan State University was cultured according to his methods. Several 2-liter aquaria with screen covers were filled with one liter of well water. A substrate of Cerophyl, trout food, and toilet tissue was blended together until lumpy and added to each aquarium in small amounts.

In the culture, a very slight aeration was used to prevent anaerobic conditions, yet leave the substrate undisturbed. The static culture was held at 21 to 25°C and had a 16-hour light photoperiod. Once a day, the emerged adults were transferred to a large screened cage which had shallow pans of water covering the bottom. After mating, the females deposited their eggs in these pans where they were easily detected and removed. Egg cases produced in this way were designated for testing or were returned to

fresh culture tanks.

Acute and subchronic Chironomus assays were performed with fourth instar larvae and second instars respectively. The second instars were easier to handle and count than first instars and did not suffer from handling mortality. All tests were conducted in a 21°C water bath using 3-liter jar exposure chambers similar to those used for Daphnia. Two one-month subchronics were terminated after two weeks of exposure due to mortality at all parathion concentrations tested. In all tests, lack of larval response to probing was interpreted as mortality.

CHEMICAL ANALYSIS AND STATISTICAL METHODS

Samples for routine water quality measurements were taken by siphoning or dipping water from the center of each tank at mid depth. Dissolved oxygen samples were takn in BOD bottles and measured with a Yellow Springs Instruments polarographic oxygen meter. Hardness, alkalinity and pH were determined by procedures contained in Standard Methods (30).

Water for parathion analyses in fish tanks was sampled in the same manner as for water quality determinations. For invertebrate tests, to avoid capture of test organisms within samples, water samples were taken directly from the diluter mixing cells and pooled. During long term tests, analyses were made weekly for all tanks with the exception of the fathead minnow chronic exposure where samples were collected daily and pooled for a weekly analysis. Analyses of samples stored for one week in amber glass bottles were not significantly different from replicates analyzed immediately. Parathion was extracted from 90 to 900 ml water samples (sample size was concentration related) in volumetric flasks by stirring with 5.0 ml pesticide-grade hexane for 15 minutes. The hexane layer was allowed to separate and was removed by pipette. Duplicate hexane aliquots were analyzed for phthalate content by gas chromatography (GC). The gas chromatographic parameters for the Hewlett-Packard 5700 or 76720 series instruments used for all analyses were:

Detector:	Ni-63, electron capture, 265°C
Injection port:	230°C
Column:	4 ft x 1/2 in, 3% OV - 17 on 80-100 mesh Chromosorb W at 200°C
Carrier gas:	10% methane in argon; flow: 50 ml per min detector purge
Injection size:	10 microliters or suitable volume
Response factors:	Parathion - 45,000 counts per nanogram Paraoxon - 20,000 counts per nanogram

System noise level (as parathion) based on hexane blanks: 0.001 to 0.02 ng of parathion on column

Identical GC conditions were used for analyses of hexane extracts of water and fish blood, and fractions of tissue extracts after clean-up.

Whole fish blood, drawn from the caudal vein, was extracted by shaking with 5 ml hexane per ml blood and analyzed by GLC after concentration to 0.5

ml without clean-up.

All other tissues were frozen at -18°C until analyzed. In the general tissue analytical procedure employed, two to three grams of tissue (wet weight) were blended with 60 gm anhydrous sodium sulfate and extracted overnight with pesticide-grade hexane (50 ml).

The sodium sulfate was filtered, washed, the hexane washes pooled (ca 20 ml) and evaporated at 60°C just to dryness. The residue was re-dissolved in hexane (ca 1 ml) and transferred to a 1-cm diameter silicic acid column (5 ml 100 mesh Mallinckrodt chromatography grade). The column was washed with 20 ml hexane and eluted with a 30 percent (v/v) benzene in hexane solution. A 25-ml benzene-hexane fraction was collected beginning at the 30 percent benzene break through (1) for low level parathion residues (in the ug/kg range). A 50-ml fraction was collected for higher parathion levels. The effluent was diluted or concentrated, as required, and analyzed by GC.

For fish tissues such as muscle, the reproducibility of analyses on replicate aliquots of the same fish was ±5 to 7 percent. Recovery of spiked samples averaged 90.8±7.6 percent based on seven tests. The range of recoveries was 83.1 to 103 percent; the coefficient of variation was 8.4 percent.

For acetylcholinesterase determinations, whole fish brains were dissected, stored up to two months in liquid nitrogen (-196°C), thawed, homogenized in phosphate buffer, and assayed by the kinetic colorimetric method of Ellman et al (38).

Statistical Methods

Whenever two or more partial kills occurred in an acute bioassay, the LC50 and 95 percent confidence limits were calculated by the graphical method of Litchfield and Wilcoxon (37). Mortalities were plotted on a probit scale against the log of toxicant concentration. A straight line was fitted to the points by the eye, and checked by the chi-square test. The LC84, LC50 and LC16 points were read from the line, and the total number of animals used between the 16 percent and 84 percent points (N) was noted. Then the confidence limits of LC50 were found as follows:

$$f = (1/2\ (LC84/LC50 + LC50/LC16)\exp(2.77/N^{1/2}))$$

$$LC50 \times f = \text{upper limit}$$

$$LC50/f = \text{lower limit}$$

Reproductive impairment of Daphnia (RI50) was found in the same way after expressing the number of offspring produced at each concentration as a percentage of the number produced in the control.

In the few cases when fewer than two partial kills occurred, LC50 values were calculated by the moving average method which does not require a straight line graph:

R_i = number of deaths at dosage i (= k, 2, 3, . . . L)
where k = lowest dosage, L = highest dosage

S_i = number of survivors at dosage i

N = number of individuals per tank

F = geometric factor between dosage levels (>1)

t = Student's t for (P = 0.05) and (N-1)(L-1) d.f.

$G = ((N(L-1)/2) - R_k - R_2 - \ldots R_{L-1})/(R_L - R_k)$

$M = \log D + \log F\ (((L-2)/2) + G)$

$$V = \frac{1}{R_L - R_k}\left[\frac{(1-G)^2 R_k S_k + R_2 S_2 + \ldots R_{L-1} S_{L-1} + G^2 R_L S_L}{N - 1}\right]^{1/2}$$

$LC50 = \text{antilog}\ (M \pm tV \log F)$

To determine differences in mean weight, length, etc., between experimental tanks, one-way analysis of variance was used, provided the variances were homoscedastic (Bartlett's Test). If a significant F value occurred, the differing tanks were found by Duncan's multiple range test. These tests are described in most general statistics texts.

Routine water quality data such as temperature and oxygen were computed as monthly means for each tank of the chronic tests. All weekly parathion analyses were used to calculate a mean, range and standard deviation (SD) of the compound concentration in each exposure tank.

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SECTION V

RESULTS

BLUEGILL

Acute Bioassay

Five preliminary and one definitive acute bioassays were performed at 22°C under previously described flow-through test parameters. The 96-hour LC50 for the definitive test was 0.51 mg/1. LC50 values for 24, 48 and 144 hour exposures were also calculated and are presented in Table 4. The sequence of toxic symptoms of acute parathion toxicity were: lethargy, dark discoloration, hypersensitivity, tremors, coughing, convulsions, extended pectoral fins and opercula, loss of equilibrium, scoliosis, hemorrhages, tetanus, and death.

Certain individual fish which succumbed during the acute test were analyzed for parathion whole body residues and the results are presented in Table 5. Incidence of lethality and time to death of analyzed bluegill varied as a function of concentration. Concentration factors calculated by dividing measured tissue parathion concentration (ug/kg) by exposure water parathion concentrations (mg/1) (Table 4) were plotted against exposure time at mortality. As shown in Figure 1, concentration factors exhibited a linear increase with exposure time indicating increased parathion bioconcentration as a function of exposure period regardless of exposure concentration.

TABLE 4. BLUEGILL ACUTE TEST

a. Number surviving per tank

Hour	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
0	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	3	2	4	5	4	3	5	3	3	4
48	5	5	0	0	4	3	3	3	5	3	3	4
96	5	5	0	0	1	1	2	1	5	2	3	2
144	5	5	0	0	1	1	2	0	5	2	3	2

b. Analyzed parathion concentration, mg/1

0	nd	nd	2.10	2.19	1.24	1.24	0.63	0.63	0.50	0.50	0.33	0.33
48	nd	nd	2.25	2.25	1.25	1.25	0.64	0.64	0.53	0.53	0.35	0.35

nd: not detectable.

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TABLE 4, continued

c. Median lethal concentrations, mg/l

Hour	LC50	Upper CL	Lower CL
24	2.25	4.35	1.16
48	1.15	1.92	0.58
96	0.51	0.72	0.36
144	0.46	0.95	0.22

TABLE 5. PARATHION RESIDUES IN WHOLE BLUEGILLS, ACUTE TEST*

Tank	Parathion in water, mg/l	Parathion in fish, mg/kg	Exposure, hours§	Concentration factor
5A	0.34	49.9	18	145
5B	0.34	61.4	24	173
4B	0.51	50.7	12	98
4B	0.51	32.5	12	63
3A	0.64	294.1	72	462
3B	0.64	197.8	70	311
2B	1.25	318.6	46	256
2B	1.25	311.6	46	250
1A	2.22	402.9	29	181
1A	2.22	430.0	29	193
1B	2.22	338.3	29	152

*See Table 4 for actual lethality data.
§Represents actual time to death.

Bluegill Chronic Test

The maximum no-ill-effect level determined for bluegill sunfish chronically exposed to parathion was between 0.17 and 0.34 ug/l based on morphological deformities evident in adults. Growth rate, spawning and fry survival were not clearly affected by parathion exposure concentrations tested up to 3.2 ug/l.

A summary of parathion analyses in water, water quality, and test temperature are given in Tables A1, A2 and A3. Bluegill survival data (Table A4) showed that parathion concentrations tested did not cause significant mortalities. Some deaths of extremely deformed fish however did occur with the majority of deaths following handling, particularly at the time of thinning. For this reason, weight measurements were not taken more frequently during the test. There were no significant differences in bluegill weights among tanks at the start of the test, at the thinning or at the end. Lengths and weights of fish at termination were approximately 17 cm, 105 gm for females, and 21 cm, 200 gm for males.

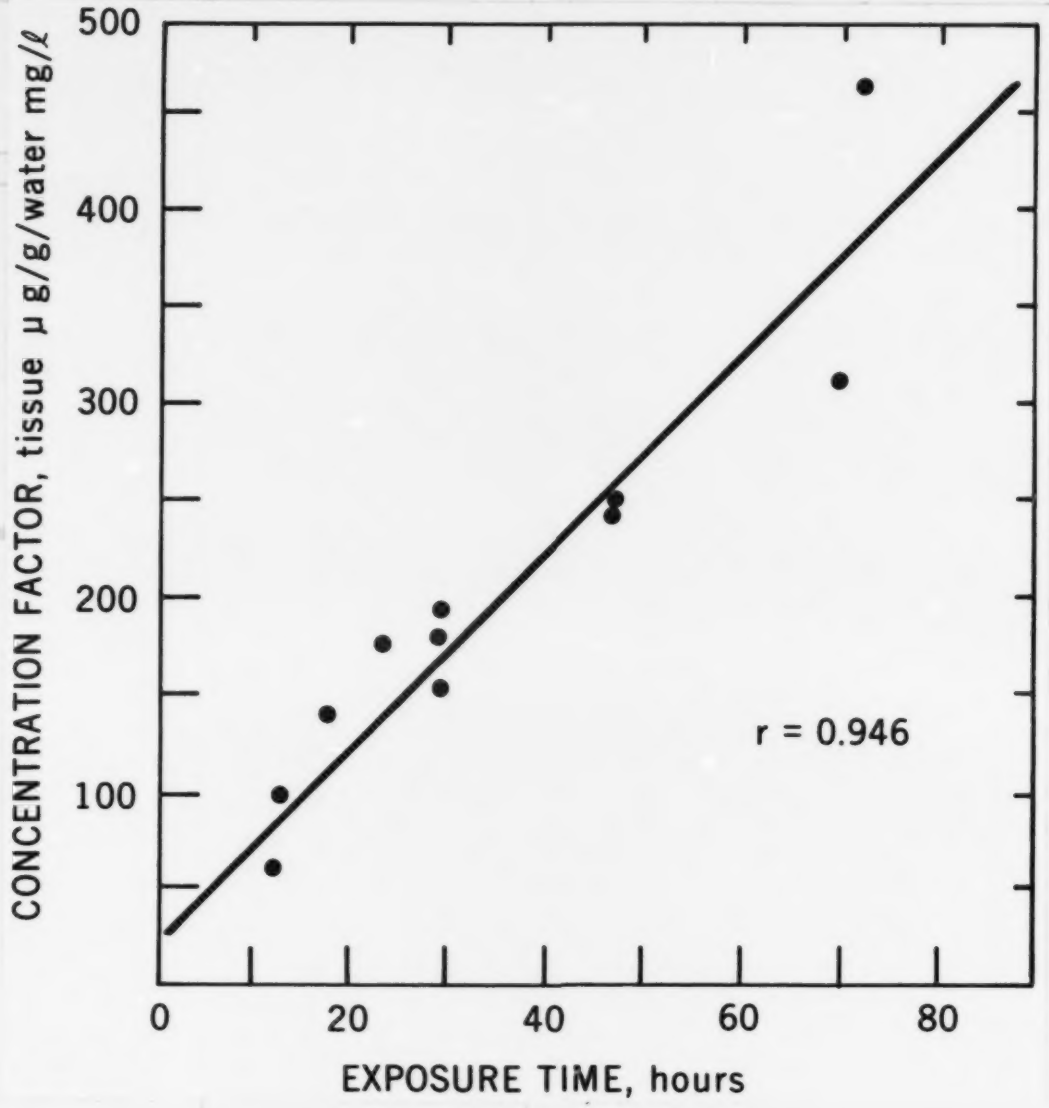

Figure 1. Concentration factors for parathion residues in whole bluegills at various exposure times. Selected whole bluegills as listed in Table 5. Exposure time is actual time to death.

Within the first four months of testing, tremors, scoliosis and extended pectoral fins were observed in the two highest exposure concentrations. In six months, protrusions in the hyal or "throat" region were noticeable at these same exposure levels. The same symptoms continued, and gradually increased in severity during the 23-month test. Table 6 lists the incidence of scoliosis and "throat" protrusions. The percentages vary to some extent between the 18 and 23 month examinations because some very deformed fish were sacrificed at thinning.

TABLE 6. INCIDENCE OF DEFORMITIES, BLUEGILL CHRONIC TEST

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Mean parathion conc, ug/l	0.06	0.06	3.23	3.14	1.53	1.59	1.00	1.00	0.34	0.34	0.16	0.17
18 months												
No. of fish	10	15	17	13	20	17	16	15	9	13	10	9
Scoliosis, %	0	13	59	38	45	24	38	33	11	0	0	0
Throat, %	0	13	88	100	70	76	75	67	11	0	0	0
23 months												
No. of fish	9	9	8	8	11	9	9	9	8	9	7	7
Scoliosis, %	0	11	25	50	18	33	22	22	0	0	0	0
throat, %	0	0	62	88*	64	100	89	89	38	56	0	0

*Loss of symptoms not explainable.

Typical protrusions in the hyal region as shown in Figure 2 were semi-rigid, vascularized masses of fatty and connective tissues extending from the branchiostegal and urohyal bone area. Scoliosis or lateral bending of the spine was usually noted posterior to the dorsal fin as illustrated in Figure 3. In bluegills, the torsion observed was both right and left laterally oriented.

Successful bluegill spawning occurred throughout the second summer. Egg production, hatchability and fry survival data are summarized in Table 7. In general, the hatchabilities improved as handling of the egg cups was reduced and the 7-day fry survival improved as more Artemia were fed per egg cup.

Although the numbers of males and females present per exposure aquarium were sufficient to provide good spawning, as illustrated below (page 24), the spawning data could not be statistically related to exposure concentration.

Growth rates of bluegill fry measured at 30, 60 and 90-day exposures were significantly different, but not related to toxicant concentration. Mean lengths and survival, Table 8, correlated inversely with numbers of fry per tank although feeding was four times daily. Regardless of the starting number of fry, each tank population fell to a few individuals at the end of

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Figure 2. Male bluegill exposed to 1.5 ug/l parathion for 23 months. Length 21 cm. Arrow indicates abnormal protrusion of fatty and connective tissue.

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Figure 3. Two adult bluegills exposed to 1.5 ug/l parathion for 23 months, showing scoliosis. Dorsal view.

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TABLE 7. BLUEGILL CHRONIC EXPOSURE TO PARATHION, SPAWNING RECORDS

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Nominal parathion conc, ug/l	0	0	4.00	4.00	2.00	2.00	1.00	1.00	0.50	0.50	0.25	0.25
Hundred of eggs spawned	5	49	20		1	90	38	105		271	26	229
	2			41	80	71	27	8		10	112	
	28		44		10	2	9	209		48	38	
	15		66		9	25	15	2				
			167		45	64	103	49				
								14				
Percent hatch, 3 days	72	46	44		49	40	80	37		57	83	86
	57		46		36	4	59	35		77	80	
	74		49		62	61	45	9		32	79	
	84		86		76	10	64	16				
			78			62	82	18				
								18				
Percent survival, 7 days*	92	56	66		68	78	0	68		32	100	22
	98		92		76	8	86	36		96	94	
	96		90		100	92	80	20		100	46	
	84		100		60	100	90	97				
			100			84	96	36				
								59				
Percent survival, 14 days*	8	10	0		0	0	0	0		0	32	2
	78		4		18	0	2	0		78	28	
	26		28		30	48	20	0		100	..	
	14		82		0	80	18	85				
						0	28	10				
								0				
Percent survival, 21 days*	6	10	..		..	..	..	..		..	28	0
	76		0		16	..	2	..		66	28	
	20		22		16	46	20	..		84	..	
	2		68		..	46	18	81				
			..			..	28	0				
								..				
Percent survival* 30 days*	6	8	..		..	..	..	..		..	.. 24	..
	76		..		16	..	2	..		64	22	..
	18		22		16	46	18	..		62	..	
	..		64		..	34	14	5				
			..			..	26	..				

*Calculated as percent of larvae hatched.

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Males	2	1	4	4	3	3	1	3	3	3	2	3
Females	7	8	4	4	8	6	8	6	5	6	3	4

the three month exposure. At 90 days, one fish in tank 1A (3.4 ug/1) had developed scoliosis.

During the first two months of the chronic, bluegill exposed to 8.0 ug/1 began to show tremors. When the bluegill were removed after 64 days exposure, their brain AChE activity was determined at 1.97±0.40 umol min/gm wet weight. This is about 11 percent of normal bluegill brain activity. Although bluegill showed signs of toxification, they were not moribund. Tables A5 and A6 show the extent to which normal AChE activity was inhibited in chronically exposed bluegill. The slightly greater AChE depression shown in Table A6 was probably caused by elevated parathion levels in exposure water during the month preceding termination (Table A1). The majority of fish in all exposure concentrations except the lowest and control appeared hypersensitive to stimuli. When startled, directional swimming response to a stimulus was almost random. A disturbance could trigger clonic (rhythmic) or alternating convulsions in some specimens.

Parathion residues in bluegill tissues exposed for 18 and 23 months are reported in Tables 9 and 10. In muscle tissue, residue levels were no greater than 25 times the exposure water concentration. Blood levels were similar or slightly higher. The unusually high tissue concentrations in Tank 3 (see Table 10) were affected by accidentally high water concentrations for two weeks before the sampling. The greatest residues were discovered in ovaries which is probably related to their high lipid content. Although the data are semiquantitative, the residues measured in spawned eggs are similar to those concentrations measured in the ovaries.

BROOK TROUT

Acute Test

Two preliminary and one definitive flow-through brook trout acute tests were completed at 12°C. The 96-hour LC50 for the definitive test was 2.00 mg/1 (Table 11). During the first 24 hours exposure of the bioassay test, total mortality occurred at the highest exposure concentration (Table A7). Many trout in the three highest exposure concentrations exhibited symptoms of lethargy, irregular breathing, and light discoloration of the skin. No symptoms of scoliosis, convulsions, or hemorrhages were visible in fish from any of the test concentrations.

The brook trout parathion 96 hour LC50 (Table 11) calculation for the definitive acute bioassay was based on mean measured exposure concentrations (Table A7) of 4.00 mg/1, 1.86 mg/1, 0./96 mg/1, 0.47 mg/1 and 0.25 mg/1 par-

TABLE 8. SURVIVAL AND LENGTHS OF BLUEGILL LARVAE

Tank	30 day		60 day		90 day	
	Number	Mean length, mm	Number	Mean length, mm	Number	Mean length, mm
0A	40	10.1	3	19.7	2	23.0
0A	9	11.3	7	14.9	3	20.7
0B	4	11.0	3	14.0	3	23.7
1A	31	10.5	13	13.2	11	18.5
1A	11	13.2	10	19.7	8	22.8
2A	8	13.6	7	23.4	5	32.4
2A	8	15.1	8	18.2	7	24.9
2B	23	10.7	6	16.8	6	22.2
3A	8	13.2	7	20.3	6	28.7
3A	12	9.4	4	12.2	3	18.0
4B	32	9.1	7	13.6	3	26.3
4B	32	8.9	4	16.2	4	20.5
5A	12	10.5	11	16.3	7	23.1
5A	12	11.5	10	14.8	4	28.8

TABLE 9. PARATHION RESIDUES IN BLUEGILLS, 18 MONTHS EXPOSURE

	Tank no. [nominal conc. parathion, ug/l]					
	0 [0]	5 [0.25]	4 [0.50]	3 [1.00]	2 [2.00]	1 [4.00]
Muscle residue*	5.4	9.8	16.9	16.0	13.1	107.0
	27.8	9.6	[illegible]6	14.2	16.1	92.4
	0.3		[illegible]	11.6	17.4	97.8
	2.9		[illegible]	13.4	10.1	90.1
	5.8			14.1	39.6	141.2
				13.5	39.9	128.4
				12.3		
Mean	8.4	9.7	14.6	13.6	22.7	109.0
SD	11.0	0.1	10.0	1.4	13.4	20.8
Blood residue*					41.4	163
					119.8	288
Ovary residue*			23.9	74	144	3436
			30.6	214	158	
				238	643	
					585	
Egg residue*	nil	...	73	552	179	813
	...	...	nil	246	134	1089
						429

*In ug/kg wet wt. Minimum detectable concentration 25 ug/kg; see "Methods."
Notes:

Recovery of spiked muscle samples (equivalent to 100 ug/kg) gave 88.4% recovery in a single test.

Precision among triplicate samples gave a coefficient of variation of 13.1%.

All blood residues except No. 1 and No. 2 sets were considered semi-quantitative since levels are at or near detectable minimum in the direct extraction samples. No evidence of paraoxon was found.

TABLE 10. PARATHION RESIDUES IN BLUEGILLS, 23 MONTHS EXPOSURE

	Tank no. [nominal conc. parathion, ug/l]					
	0 [0]	5 [0.25]	4 [0.50]	3 [1.00]*	2 [2.00]	1 [4.00]
Muscle residue	See note **					
Blood residue	0.9	0.6	6.8	253.0	37.5	133.7
	1.2	1.8	9.4	118.5	42.7	159.6
	2.1	1.5	8.1	62.9	56.0	125.3
	<0.1	3.9	25.7	78.7	61.7	123.7
	<0.1	3.8	3.7	104.3	51.0	131.4
	2.0	4.0	8.5	97.8	67.2	164.2
	<0.1	4.1				157.3
		9.4				111.0

*Mean parathion concentration for last month of test was accidentally high: 2.5 ug/l (Table A1).

**Muscle residue values all at or below reliably detectable levels (≈25 ug/kg). Precision at these low levels is estimated at about ±50%.

TABLE 11. BROOK TROUT 96 HOUR ACUTE LC50, mg/l

LC50	Upper CL	Lower CL
2.00	3.03	1.32

Determined by Litchfield and Wilcoxon method from data in Table A7.

athion. Use of the Litchfield and Wilcoxon method resulted in a 96 hour LC50 for brook trout of 2.00 mg/l parathion.

Trout muscle parathion residues determined for mortalities from the definitive acute bioassay are presented in Table 12. Incidence of lethality and time to death varied as a function of concentration. The concentration factors presented in Table 12 are generally a function of exposure period (time to death) and indicate a slight increase in residue concentration factor to 140 hours exposure followed by a decline (144 hours). Actual tissue concentrations (mg/kg) were seen directly proportional to exposure concentration (mg/l). Appendix B presents details of separate investigations concerning the toxicity and bioconcentration characteristics of parathion to the brown trout (Salmo trutta). Results indicate a substantial agreement to data obtained for brook trout.

Brook Trout Chronic Test

Over a nine month exposure period, adult brook trout chronically exposed to various parathion concentrations as high as 7 ug/l showed no significant differences in growth rate, maturity, or spawning success. However, relatively high residue concentrations of parathion were measured in the blood, muscle and other tissues of brook trout chronically exposed to the parathion concentrations tested.

TABLE 12. PARATHION RESIDUES IN BROOK TROUT MUSCLE, ACUTE TEST

Exposure time to death, hr	Mean water conc, mg/l	Muscle conc, mg/kg wet weight*	Concentration factor
8	3.18	217	68
8	3.18	346	109
114	1.86	165	89
114	1.86	215	116
144	0.53	121	228
144	0.53	83	157
140	0.27	70	259
140	0.27	93	344

*Each analysis from an individual fish.

Water quality parameters, temperature and parathion analyses data for the chronic exposure are given in Tables A8, A9 and A10. Most mortalities which occurred during the test (Table A11) were attributable to disease (fungus or furunculosis) or to accidental test system mechanical failure. At no time during chronic exposure did trout exhibit symptoms typical of acute organophosphate poisoning such as tetany or convulsions. Fish weight measurements made at the start of testing and after 4 and 9 months of exposure (Table A12) indicate uniform weight gains for trout in all exposure concentrations. Although no significant differences between mean weights were found, weight variability between individuals was attributable to biomass (density) differences between exposure tanks.

Fish discarded from the chronic at the time of thinning (six months exposure) were analyzed for parathion residues in blood, muscle, and other tissues and for acetylcholinesterase activity. The blood residues (Table 13) were plotted against the analyzed parathion exposure concentration in water at the time of removal (Figure 4).

TABLE 13. PARATHION IN BROOK TROUT BLOOD, SIX MONTH EXPOSURE, INDIVIDUAL FISH, ug/l

	Tank							
	0A	1A	2B	3A	4A	4B	5A	5B
	6.2	1007	737	647	113	153	68	79
	63.7	1597	885	695	96	151	24	58
	10.6		1139	726	96		61	69
	40.5		776	337	93		52	103
	0.5		662	515			101	80
	6.9		528	654			42	50
				649			68	110
							50	
Mean	21	1302	788	603	100	152	58	78
SD	21	417	208	136	9	1	23	24

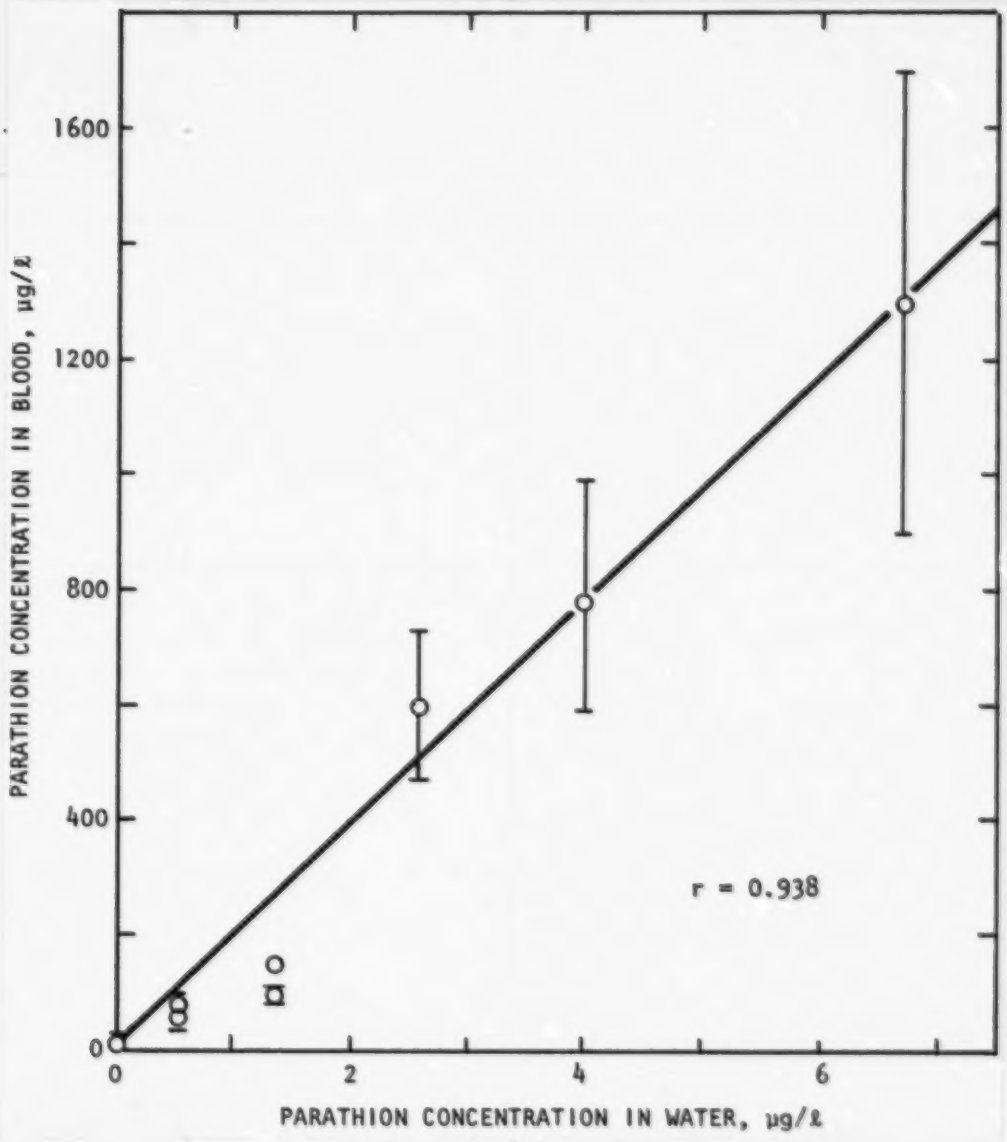

Figure 4. Parathion in brook trout blood vs. parathion in water, six month exposure.

A least-squares line fitted to the data has the equation:

$$C_b = 207C_w - 39$$

where C_b is parathion concentration in blood and C_w the parathion concentration in water. Results indicated that trout concentrated parathion approximately 200 times over exposure water concentrations.

The parathion residue levels for muscle and other tissues show equally large concentration factors (Table 14). Muscle residue was plotted against water concentration in Figure 5 and fitted to a curve by eye. If treated as a straight line, this has an equation of:

$$C_m = 553C_w - 176$$

No paraoxon was detected in any of the analyses despite the use of solvents to extract it from the clean-up columns. The parathion residues reported in Table 14 were the highest concentrations found in any chronic exposure. Brain acetylcholinesterase activities of the same fish (Table 15) were plotted against exposure concentration. Figure 6 shows that about 5.6 ug/l of parathion was required to depress trout brain AChE activity to a 50 percent of normal level. However, no gross observable symptoms of central nervous system toxification were noted in fish exposed to the highest parathion concentration tested.

TABLE 14. PARATHION RESIDUES IN BROOK TROUT TISSUE, SIX MONTH EXPOSURE, ug/kg WET WEIGHT

	Tank no. [water conc, ug/l]						
	0A [0.03]	1A [6.7]	2B [4.0]	3A [2.6]	4B [1.4]	5A [0.6]	5B [0.6]
Muscle	nil	4454[a]	1958	1258[b]	176[a]	244	171[a]
	18	3229[a]	1820[a]	1411[b]	474[b]	130	139
	12		1874	921	606		
	nil			998			
				1124			
Kidney	nil	2942	998	1245	123		nil
	125	556	815	1051	nil		277
			3332				
Gill	nil	5954	2503	1932	492	...	nil
	26	4090	2430	2659	516		160
			3294				
Gonad	nil	2208	2136	2050	175		nil
	16	1344	414	1284	11		176
			579				

All single samples except (a) average of duplicate samples and (b) average of triplicate samples. Reagent blank = 3.0 ug/kg. Recovery at 1000 ug/kg level averaged 9.08±7.6% (coefficient of variation = 8.4%); range 83.1 to 103% for seven tests. All values uncorrected for percent recovery.

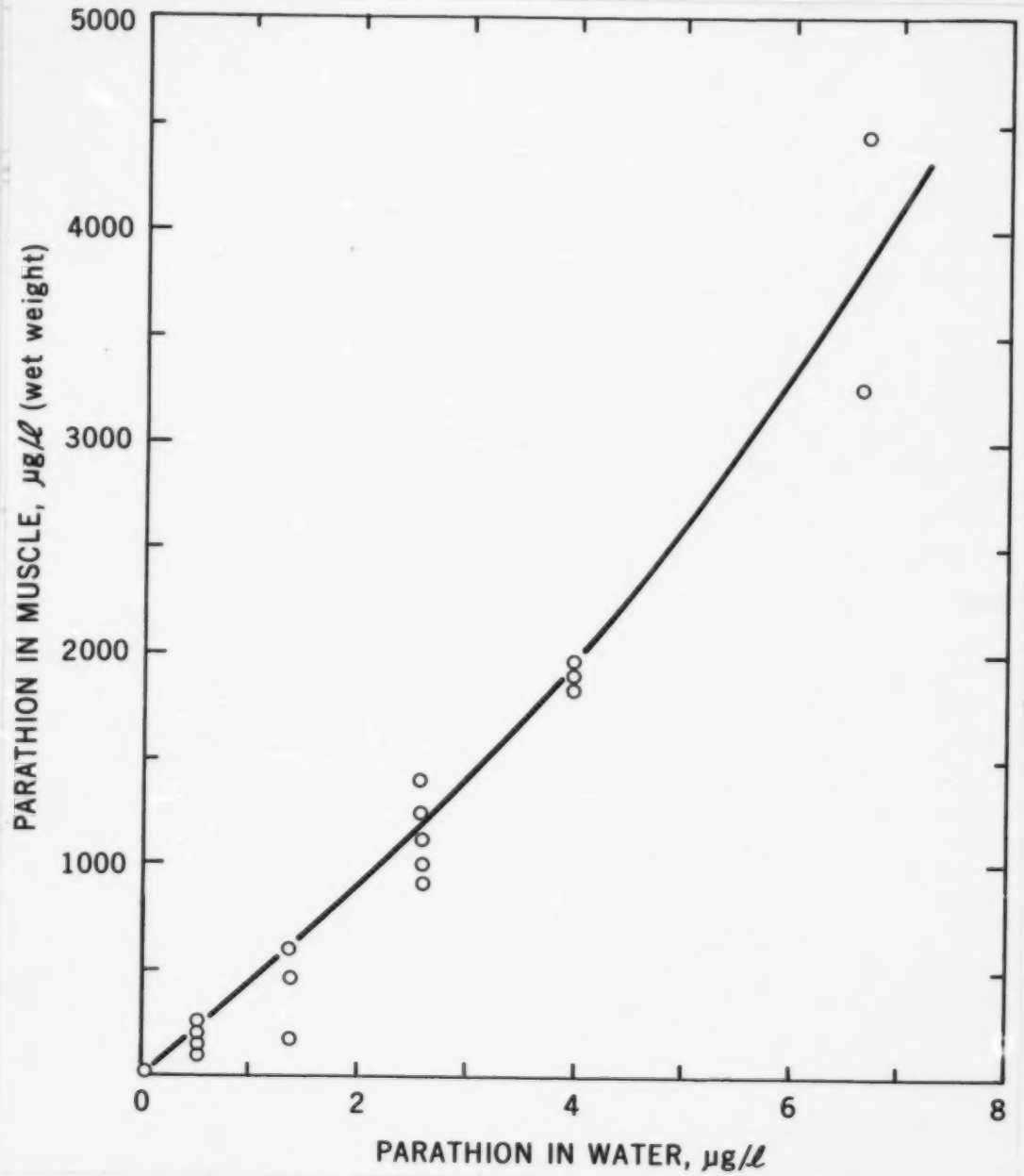

Figure 5. Parathion in brook trout muscle vs. parathion in water, six month exposure.

TABLE 15. BRAIN ACETYLCHOLINESTERASE ACTIVITY OF BROOK TROUT SIX MONTH EXPOSURE, INDIVIDUAL FISH, umol/min/gm

	Tank							
	0A	1A	2B	3A	4A	4B	5A	5B
	6.60	3.79	5.22	4.19	4.96	4.28	5.51	6.72
	6.49	1.88	3.44	4.42	4.65	4.59	4.96	6.49
	6.63		5.34		5.85		5.85	
	6.66		4.16		6.03		6.72	
	5.85						5.74	
	6.57						6.49	
							4.71	
Mean	6.47	2.84	4.54	4.30	5.37	4.44	5.71	6.60
SD	0.31	1.35	0.90	0.16	0.67	0.29	0.74	0.16

Brook Trout Spawning

Brook trout in the chronic exposure spawned between December 23 and January 15 test dates. A summary of the number of spawnings, egg production and survival of embryos and larvae is given in Table 16. Only a very small percentage of embryos were deformed and these numbers could not be correlated to exposure concentrations. Egg hatchability was uniformly poor but was felt a function of handling damage and elevated water temperature (13°C) during spawning. Exposure water temperature in excess of the optimal 9°C was felt to have delayed maturation and inhibited spawning activity (39). To document maturation, the gonad weights of the discarded fish were taken at the time of thinning (Table A13) and at the test termination (Table A14). The gonad weights divided by the fish's total weight produced a gonadosomatic index expressed as a percent. The weights shown in Table A14 are somewhat biased since small and immature fish were selectively discarded. For both males and females, the development was variable and appeared unrelated to toxicant concentration. The gonadosomatic indexes determined at termination show that spawning was not complete. Female gonadosomatic indexes greater than 1.00 represent loose eggs in the body cavity which were never released (Table A14).

To supplement the chronic hatchability data, nineteen-day-old brook trout eggs were incubated at 10°C in three concentrations of parathion until hatched. The following percent survivals to hatch were observed:

Control	10 ug/l	32 ug/l	100 ug/l
77.5%	52.5%	41.5%	38.4%

Eggs in the 32 and 100 ug/l solutions exhibited poor development and more transparent chorions than controls. Some of the eggs in both concentrations showed cerebral development without corresponding spinal development. The lower embryo survival at the two highest levels partly resulted from premature hatching. Although the hatchability at 10 ug/l was less than the control group, development appeared to be normal.

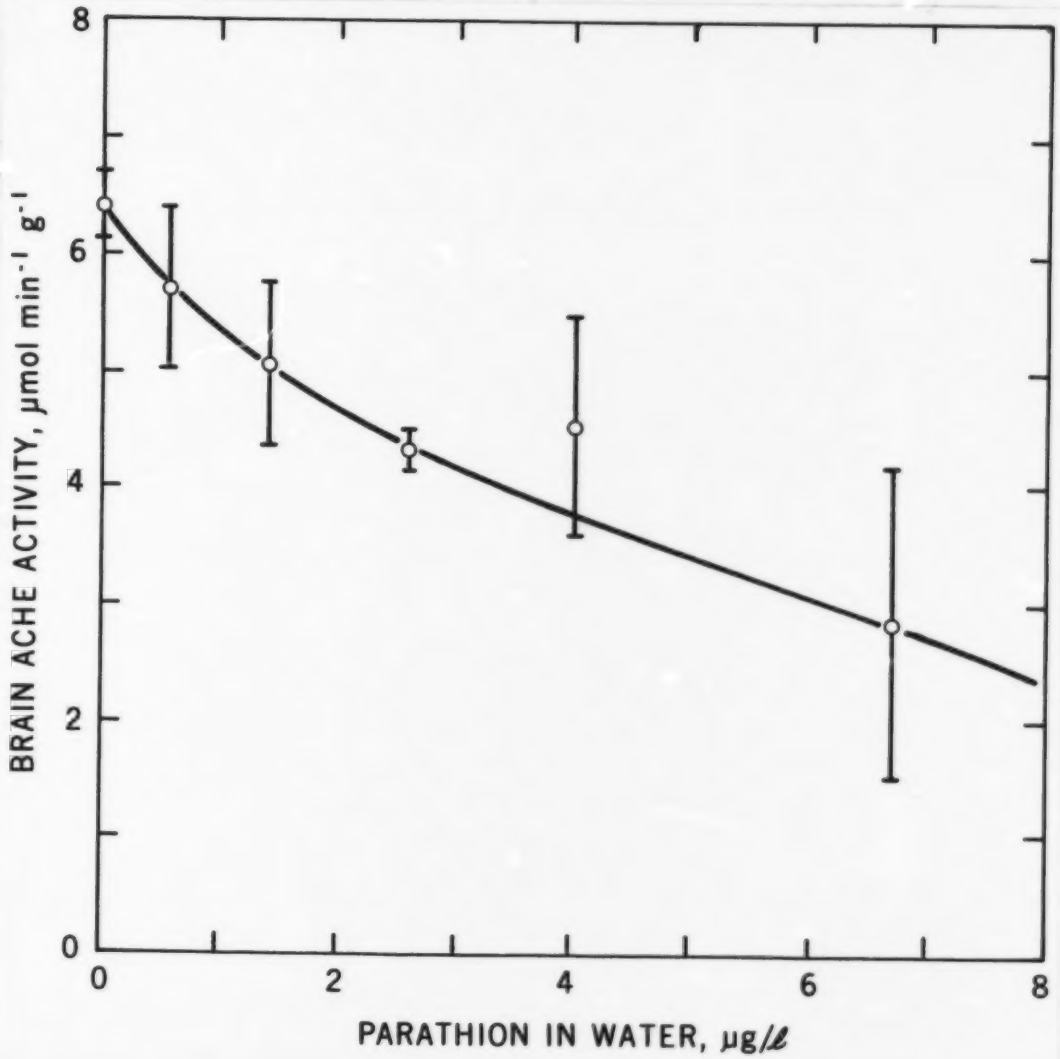

Figure 6. Brook trout brain acetylcholinesterase activity vs. parathion concentration in water, six month exposure.

TABLE 16. BROOK TROUT SPAWNING RECORD

	Tank								
	0A	1B	2B	3A	3B	4A	4B	5A	5B
Number spawnings	1	2	3	2	2	2	3	7	1
Total eggs spawned	360	1249	787	716	924	929	1607	479	48
12 day viability, percent of spawn	26	85 42	2	76 56	15	100	78 0	24	
Total hatchability, percent of spawn	6	12	20	12	0	6	22	4	0
30 day fry survival, percent of hatch	0	0	10	67	0	67	18	0	0

Note: overall spawning and hatchability variability attributable to external variables of handling stress and temperature, not to parathion concentration.

Adult Brook Trout Termination

Chronically exposed adult brook trout were sacrificed on February 6, three weeks after the last spawning. Blood obtained from 34 trout representing all concentration groups was analyzed for parathion residues as was muscle tissue from 31 exposed fish. Residue analyses for parathion were also done on kidney, gill, gonad and gastrointestinal tract samples from each of two to three fish in each exposure concentration. Brain AChE was determined for 39 fish.

Tissue residue analyses results, including blood levels, are tabulated in Table 17. Blood parathion concentrations versus exposure water concentrations are plotted in Figure 7. Trout blood and tissue parathion residue levels measured after nine months exposure were lower than corresponding values determined on fish sacrificed after six months exposure.

Several of the adult trout from each parathion concentration group were exposed to flowing water without toxicant for 48 and 168 hours. Blood and muscle parathion residue analyses on these fish are shown in Table 18. Brain AChE determinations were also performed and results are given in Table 18. Data indicate that AChE inhibition was essentially irreversible over the time studied (168 hours). The blood showed a rapid loss of parathion within 48 hours and a subsequent slower rate of decrease while muscle parathion concentrations decreased more slowly than blood. The limited samples available and the large variances among individual fish make it difficult to quantify "wash-out" rates but the blood and tissue parathion uptake concentration appears to be readily reversible.

Some general conclusions may be drawn on the basis of the six and nine month analyses. Parathion levels in blood correlate with water concentrations at the time of sampling. Blood levels appear to rapidly reflect exposure levels in the water. Tissue residues also correlate with water concentrations; however, tissue levels will change less rapidly with change in water concentrations. High correlation also exists between blood parathion

TABLE 17. BROOK TROUT PARATHION RESIDUES AND AChE, 9 MONTHS

Tank	Parathion in water, ug/l	Parathion, ug/kg wet weight						AChE, umol/min/g
		Blood	Muscle	Gill	Kidney	Gonad	GI tract	
0A	0.01	23	0.8					3.97
		42	0.6	3.1	0.2	<0.01	0.7	5.04
		94	1.9					5.12
		23	1.0					3.01
0B	0.01	58	0.6	1.4	0.1	...	28	4.49
		33						5.77
		29	0.1					4.62
		20						4.19
1A	8.30	733	1220	3172	400	1812	8989	3.44
		1021	399					2.79
		990	4157*					2.15
1B	8.72	625	1623					3.17
		743	876	1727	217	939	160	4.39
		720	2181**	1143	614	1780	11598	3.86
2A	5.53	361	297					6.24
		355						4.45
		...	671	2358	311	1054	5666§	5.91
2B	4.24	337	318	1483	374	705	200	4.46
		446	...					5.21
		304	...					6.17
		...	413*	1085	78	519	352	4.71
3A	2.86	184	270					4.30
		252	189**	703	192	6	1672	4.81
		482						4.35
		166	319**					5.44
3B	2.76	232**	272	959	183	407	1105	4.42
		217**	511§	1083§	774	15048§	1210	4.76
4A	1.45	114	7	179	11	36§	106	6.24
		111	118					6.06
4B	1.26	141						5.85
		87	44	265	53	...	110	6.00
		82	34					4.36
5A	0.53	41	33	138	16	69	135§	5.63
		40	58**					5.73
		52						5.27

*Analysis done immediately after fish was sacrificed; all other samples were stored at -18°C for 2-1/2 months.
**Duplicate samples average.
§Probable interference contribution to parathion value.

continued

TABLE 17, continued

Tank	Parathion in water, ug/l	Parathion, ug/kg wet weight						AChE, umol/min/g
		Blood	Muscle	Gill	Kidney	Gonad	GI tract	
5B	0.44	39	36					6.51
		33						5.65
		36	73	96	63§	38	28	5.56
		63						5.45

*Analysis done immediately after fish was sacrificed; all other samples were stored at -18°C for 2-1/2 months.
**Duplicate samples average.
§Probable interference contribution to parathion value.

concentrations and tissue parathion concentrations when measured after chronic exposure (six or nine months); the correlation coefficient for muscle parathion versus blood parathion was 0.967 ($p < 0.001$).

The high variance in parathion water concentrations measured on a weekly basis during chronic exposure probably accounts for the differences in tissue residue concentrations observed at six and nine months. Since parathion appears to be reversibly taken up and released, measured residue levels reflect the immediate past exposure conditions.

Brain AChE levels, as expected, exhibited an inverse dependence on water concentration. The degree of inhibition was less marked than with bluegills. Essentially the same AChE effects were found at six and nine months. Relatively high variance within the concentration groups and especially the control group was found. However, the highest concentration group exhibited AChE levels significantly lower than the other experimental groups (excluding the control group) ($p = 0.05$)

FATHEAD MINNOW

Acute Test

A preliminary static acute test conducted at 22°C using adult minnows gave the following results based on measured initial parathion concentrations:

LC50 - 24 hr = 2.28 (2.92 - 1.77) mg/l
48 hr = 2.02 (2.64 - 1.55) mg/l
96 hr = 1.60 (2.05 1.25) mg/l

A definitive flow-through acute bioassay was conducted at 24°C using adults (approx. 2.2 gm each). The results of this bioassay are reported in Table 19.

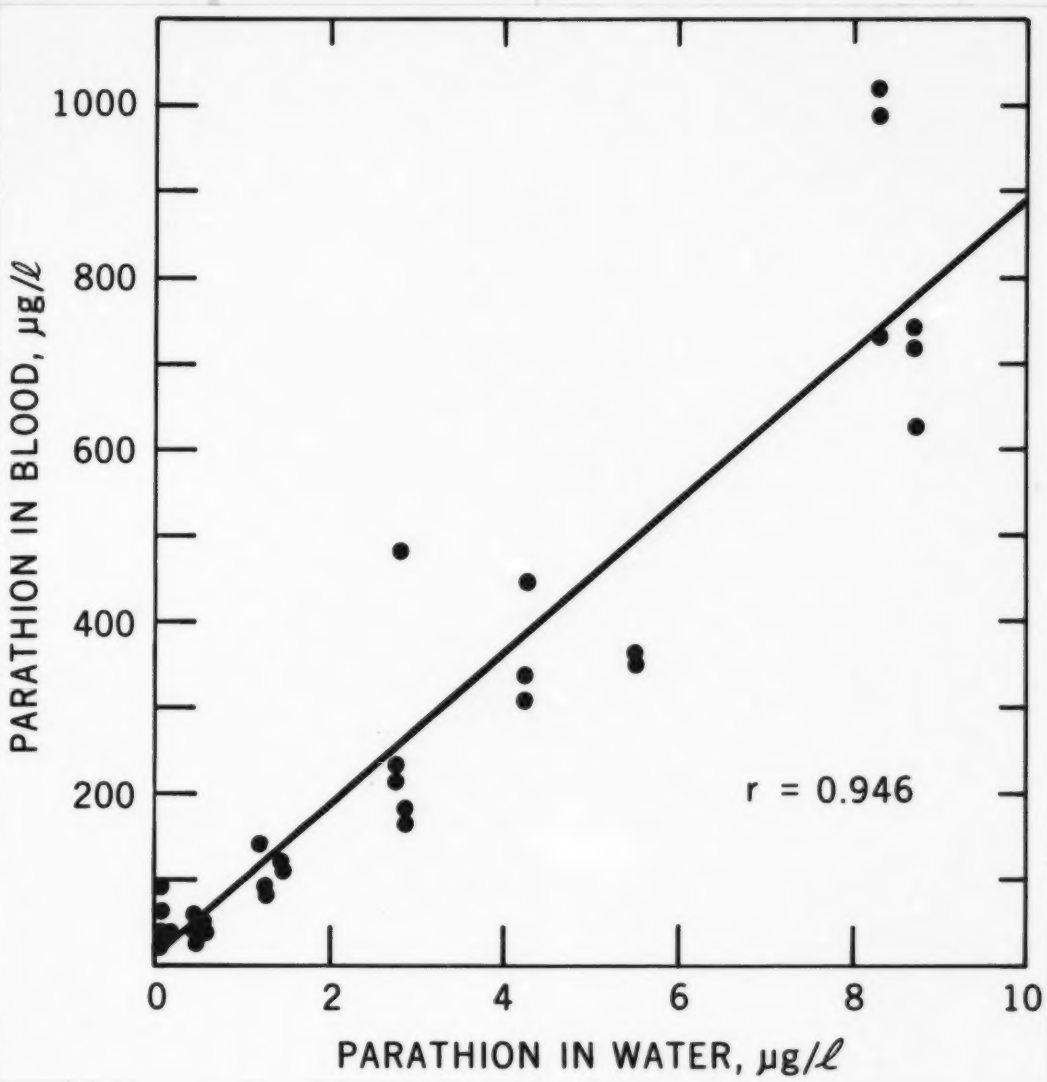

Figure 7. Parathion in brook trout blood vs. parathion in water, nine month exposure.

TABLE 18. BROOK TROUT PARATHION RESIDUES AND AChE, RECOVERY

48 hour elution in fresh water				168 hour elution in fresh water			
Tank	Blood residue, ug/l	Muscle residue, ug/kg	AChE, umol/min/g	Tank	Blood residue, ug/l	Muscle residue, ug/kg	AChE, umol/min/g
0A	27	35	5.42	0A	...	17	4.31
0B	25	41	4.99	0B	19	...	5.74
1A	507	587	4.45	1A	293	942	4.78
1B	396	634	4.08	1B	422	1124	3.62
2A	223	478	4.09	2A	196	365	4.91
2B	229	161	4.00	2B	127	462	
3A	182	187	4.48	3A	65	223	4.23
3B	364	290	4.30	3B	40	58	5.41
4A	94	92	5.77	4A	35	60	4.59
4B	144	74	8.48	4B	84	186	4.02
5A	123	85	6.11	5A	21	62	9.08
5B	57	75	3.84	5B	23	128	4.88

TABLE 19. FATHEAD MINNOW ACUTE TEST, 24°C

a. Number surviving

	Tank									
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	0	0	0	0	9	6	9	8
48 hr	10	10	0	0	0	0	7	1	6	4
96 hr	10	10	0	0	0	0	6	1	6	4
Parathion*	nil	nil	5.31	5.88	1.92	2.24	1.04	1.04	0.53	0.49

*Mg/l, means of four analyses.

b. LC50 values, mg/l

Hour	LC50	Upper Cl	Lower Cl
24	2.20	3.90	1.24
48	0.50	1.03	0.24
96	0.50	1.03	0.24

Fathead Minnow Chronic Exposure

The fathead minnow chronic exposure resulted in a maximum acceptable parathion concentration of approximately 4 ug/l. This value was defined on exposure concentration related incidence of adult deformities and reproductive impairment. Data on water quality, temperature and parathion concentrations for the chronic exposure are summarized in Tables A16, A17 and A18.

For the first 30 days of parathion exposure, minnow fry showed good survival and growth (Table A19). There were no significant weight differences between exposure tanks. At 60 days, the mean length in Tank 3B was significantly less ($p = 0.05$) than tanks 3A, 5A and 5B but not different from any others (Table A19). During the third month of exposure, symptoms of toxification began to become evident in several of the higher exposure concentrations with convulsions and scoliosis observed occasionally. By time of spawning, there were deformities (including scoliosis, lordosis and abnormally shaped heads) in almost half of the fish in the 50 ug/l tanks (Table 20). Weights, however, were not significantly different between tanks, as determined by analysis of variance.

TABLE 20. MINNOW ADULT WEIGHTS AND DEFORMITIES, 8.5 MONTHS

	Females		Males			Parathion exposure conc, ug/l			
Tank	Number	Mean wt, gm	Number	Mean wt, gm	Percent deformed	Mean	SD	High	Low
0A	8	1.15	4	2.30	0.0	0.14	0.14	0.82	nil
0B	9	0.98	4	1.88	0.0	0.12	0.18	0.82	nil
1A	11	1.05	2	1.92	46.2	49.40	18.08	104.20	8.61
1B	9	1.16	2	1.38	45.4	48.53	15.06	104.20	16.81
2A	7	1.29	3	1.78	30.0	21.51	8.16	44.86	3.19
2B	5	1.32	2	1.46	28.6	21.94	7.18	44.86	6.70
3A	6	1.25	2	1.88	0.0	15.61	7.55	38.70	2.06
3B	8	1.07	4	1.99	50.0	15.36	7.40	38.70	3.34
4A	9	0.94	4	1.73	15.4	8.95	4.14	17.90	2.81
4B	7	1.09	1	1.75	25.0	9.02	2.87	17.90	2.24
5A	8	1.22	5	1.99	7.7	4.46	1.55	7.30	1.13
5B	5	1.23	8	1.90	0.0	4.36	1.18	7.30	1.60

Spawning first occurred in the controls followed by spawning in the other exposure concentrations several days later. Total egg production (Table 21) and the number of spawns per female during the reproductive phase of the chronic exposure were highest in the two control tanks. In relation to the control (normal) series, all parathion exposure concentrations tested exhibited reduced or impaired reproductive capacity. Egg hatchability and 30-day fry growth and survival data could not be related to parathion exposure concentration.

TABLE 21. MINNOW SPAWNING RECORD

	Total eggs spawned	No. of spawnings	spawns per female	% Mean hatch	Spawns hatched	30-day survival, percent	Mean length, cm
0A	2096	14	1.75	72.4	9	70.0	0.98
0B	3591	21	2.33	88.2	19	42.5	1.49
1A	463	8	0.73	65.5*	3		
1B	51	1	0.11	42.0	2	40.0	1.20
2A	251	2	0.29	...	0		
2B	979	7	1.40	44.4	5		
3A	103	3	0.50	...	0		
3B	397	5	0.62	51.2	6		
4A	2205	12	1.33	74.3	14		
4B	0	0	0.00	...	0		
5A	570	5	0.62	75.0	6		
5B	399	4	0.80	65.5	4	12.5	1.68

*Four sets of eggs spawned in 0B were hatched in 1A - 70% mean hatchability.

Whole minnows were analyzed for parathion during the test but because of their small size were pooled. Fish taken at 70 days exposure (thinning) had lower residue concentrations than fish samples at later sample points (Table 22). The mean concentration factor for 260-day chronically exposed minnows was approximately 115--less than trout but higher than bluegills.

TABLE 22. PARATHION RESIDUES IN WHOLE FATHEAD MINNOWS, ug/kg

Days exposure	Water parathion concentration, ug/l					
	0.15	49.0	21.7	15.5	9.0	4.2
70	...	1040(20)	615(2)	251(19)	214(22)	256(8)
82-138	...	9975 (3)	4400(5)	2256 (3)	1796 (1)	1411(4)
260	14(2)	8300 (2)	2270(2)	510 (2)	601 (2)	846(2)

Numbers in parentheses are numbers of fish pooled.

DAPHNIA MAGNA

The acute and chronic LC50 values for parathion determined by exposure of Daphnia magna are summarized in Table 23. Representative test data are presented in Tables A20 and A21. The lethal response of daphnids to parathion occurred within narrow test concentration limits. Thus, the MATC of 0.08 ug/l was very near the three-week chronic LC50 of 0.14 ug/l. Symptoms of parathion toxicity observed in daphnids during the acute exposures were an erratic circular swimming motion, and later, inactivity and immobility.

The parathion concentration needed to reduce reproduction by 50 percent (RI50) was greater than the chronic LC50 value. This occurred because young were produced during the third week of exposure at concentrations that later killed the adults (Table A21).

TABLE 23. PARATHION TOXICITY TO Daphnia magna

a. Acute LC50, ug/l

24 hour	48 hour	96 hour
2.70 (5.61 - 1.30)	1.00 (1.73 - 0.58)	0.62 (0.90 - 0.43)
3.21 (5.10 - 2.02)*	1.27 (1.63 - 1.09)*	0.65 (0.88 - 0.48)*

b. Chronic LC50, ug/l

1 week	2 week	3 week	RI50
0.28 (0.29-0.26)	0.25 (0.22-0.28)	0.14 (0.16-0.13)	0.24 (0.32-0.17)
0.56 (0.85-0.37)	0.38 (0.70-0.21)	0.19 (0.30-0.12)	0.48 (0.87-0.26)
0.35 (0.47-0.26)			
0.37 (0.74-0.18)			
0.45 (0.55-0.37)			

*Static test at 22°C; all other tests flow-through at 18±°C.
MATC = 0.08 ug/l.

The MATC of 0.08 ug/l (Table A21) was 0.08 times the 18-hour LC50 of 1.00 (Table 23) and 0.13 times the 96-hour acute value (Table 23). The MATC was considered to be the highest concentration tested in which the number of young produced did not differ significantly from controls, as determined by analysis of variance.

For residue analyses, 0.4 to 20 mg of parathion-exposed Daphnia were collected, filtered and air dried. Hexane extracts were cleaned with a silicic acid column and analyzed by GC. No evidence of significant parathion residues could be detected above the GC background noise for any of the sublethal exposure levels. The possibility of decomposition of parathion during the air drying process may have contributed to the low residues detected.

GAMMARUS FASCIATUS

Results of parathion acute and chronic exposure using G. fasciatus as the bioassay organism are reported in Table 24. Two long-term (subchronic) tests were terminated when survival in the lowest concentration fell below 50 percent of the survival in the controls. Table A23 shows one of these chronic tests in which the controls and low levels began reproducing. Table A22 presents data on acute parathion exposure of G. fasciatus for a 96-hour period. The mean of all acute tests resulted in a 96-hour LC50 of 0.39 ug/l.

CHIRONOMUS TENTANS

The C. tentans results summarized in Table 25 show a striking change in LC50 with length of exposure. Between acute and chronic periods the lethal concentration varied over three orders of magnitude. Preliminary static

acute tests using tissue paper substrate were discarded when it was discovered that parathion was removed by such substrates from the water in several days. The flow-through tests eliminated this problem of decreasing parathion concentration. Data related to C. tentans acute and subchronic parathion exposures are presented in Tables A24 and A25 respectively.

TABLE 24. PARATHION TOXICITY TO Gammarus fasciatus

LC50, ug/l			
24 hour	48 hour	96 hour	Temp, deg C
2.1 (3.8-1.2)	0.62	0.43 (0.65-0.29)	20
1.27 (1.62-0.99)	0.82 (0.99-0.68)	0.62 (1.16-0.33)	19
2.3	0.86 (1.23-0.60)	0.26 (1.00-0.07)	20
...	...	0.25 (0.29-0.21)	20

MATC probably greater than 0.04 ug/1.

TABLE 25. PARATHION TOXICITY TO Chironomus tentans

Exposure, days	LC50, ug/l	Instar at start
1	660 (1040 - 420)	4th
2	135 (703 - 26)	4th
4	31.0 (43.4 - 22.1)	4th
5	7.3 (11.8 - 4.6)	2nd
8	2.2 (3.2 - 1.5)	2nd
14	2.3 (3.4 - 1.6)	2nd
14	2.9 (3.7 - 2.3)	2nd

SECTION VI

DISCUSSION

Of those fish species tested, bluegills were most sensitive to parathion toxicity. The 96-hour flow-through acute LC50 of 0.51 (0.72-0.36) mg/l determined for juvenile bluegills agrees with the 0.58 ppm value reported by Pickering, Henderson and Lemke (40) for large bluegills in soft water. These authors also reported a much lower parathion LC50 concentration of 0.095 ppm for small bluegills under static conditions.

Yearling brook trout exhibited a 96-hour LC50 of 2.00 (3.03-1.32) mg/l under flow-through conditions. This is, however, somewhat greater than the 72-hour LC50 of 0.92 mg/l given by Leland (16) for rainbow trout. It was noted that trout responded over a wider range of parathion concentrations than either bluegill or minnow.

Adult fathead minnows were killed by parathion concentrations within the range of concentrations also lethal to bluegill and trout. A preliminary static acute test produced a 96-hour LC50 of 1.60 (2.05-1.25) mg/l, while a flow-through acute LC50 of 0.50 (1.03-0.24) mg/l was realized in 96 hours. The preliminary static acute result (1.64 mg/l) agrees with literature 96-hour LC50 values of 1.3 mg/l (40), 1.4 mg/l (22, 41) and 1.6 mg/l (42). By comparison, the flow-through results may seem low; however, all test results were confirmed in preliminary testing, fish were handled carefully to avoid stress and parathion concentrations were checked by independent analysis.

Chronic parathion effects on bluegill sunfish, including scoliosis, abnormal protrusion of branchiostegal tissue and depressed acetylcholinesterase activity, were found at parathion concentrations above 0.17 ug/l. Scoliosis was found under similar conditions to involve calcium deposition, and fusion of two to four vertebrae (Carter, 14). The protrusion in the "throat" area of many parathion-exposed bluegills was unexpected. Affected fish had shallow rapid breathing and flared gill covers--presumably due to parathion-related nerve damage. The observed protrusions may have resulted from mechanical damage to the fish's branchiostegal area as a result of such spasmodic buccal pumping. Further histological characterization would help to determine the cause of such parathion-related tissue abnormalities.

At a 3.2 ug/l parathion concentration bluegills were able to live having only 16 percent of normal AChE activity. Under optimum laboratory conditions and little disturbance, affected fish could function marginally. In nature, however, the ability of these same affected fish to feed and escape predators would be diminished due to natural stress.

Brook trout chronically exposed to 7.2 ug/l parathion for 9 months exhibited no parathion-related toxicity symptoms, as judged by weight gain, external appearance, AChE activity and production of embryos. The data obtained for egg and fry survival during the chronic study are not conclusive although a level of 32 ug/l parathion reduced hatchability of embryos from untreated parents, while a level of 10 ug/l did not.

Fathead minnows exhibited deformities and a reduced egg production during chronic exposure to parathion concentrations above 4 ug/l. No reductions in growth rate, hatchability or fry survival were demonstrated at the higher concentrations tested. Thus, in both the bluegill and minnow tests, the juvenile and adult stages seemed more sensitive to parathion than either eggs or larvae. Although this could simply be a function of length of exposure, Lewallen (43) found that 2.0 mg/l parathion had no effect on one-week-old rainbow trout sac fry, while 70 percent of one-month-old fry were killed at the same level in 24 hours. Because parathion toxicity depends on activation, it is possible that older animals metabolize parathion more efficiently than young ones, increasing its toxic effect.

The retention of parathion in blood, muscle and other tissues was demonstrated for all three fish species tested. Bluegill and trout acutely exposed to parathion had residues several hundred times greater than exposure water concentrations. In chronic tests, where residues probably reach a steady state, brook trout demonstrated the highest parathion tissue residues, including muscle tissue with a concentration factor of 533. Whole minnows had concentration factors of about 200 while bluegills had a maximum parathion concentration of approximately 25. This suggests that bluegills metabolize parathion more readily than either trout or fathead minnows which could explain their greater parathion sensitivity. We cannot rule out the possibility that the toxicity effect might be temperature as well as species dependent. Temperatures during exposures were increased from trout, to minnow, to bluegill. It is possible that bluegills held at lower temperatures might metabolize less, and store more parathion. Further metabolic pathway studies in fish or in other cold-blooded animals should be designed to relate effects of temperature, rate of metabolism and proportions of major metabolites.

The two crustaceans tested, D. magna and G. fasciatus, were more sensitive to parathion than either the insect, C. tentans, or the fish species tested. For Daphnia, the maximum acceptable chronic level, about 0.08 ug/l, was 0.13 times the 96-hour acute level of 0.63 ug/l. The acute value is close to 0.8 ug/l reported by Priester (44) (50 hours) and Boyd (45) (26 hours). Gammarus, with a mean 96-hour LC50 of 0.39 ug/l, had a chronic effect concentration much less than 0.04 ug/l, a lethal level in one month. At such low effect levels (which approach the chromatographic background for routine work) it became impractical to carry out meaningful tests for more than one month. Since crustaceans such as Daphnia and Gammarus are typical foods for many small fish, parathion levels in natural waters should be limited to considerably less than 1 ug/l. In terms of protecting food chain organisms, and hence fish, this would not be an overly conservative level.

The midge, C. tentans, had a wide range of sensitivities to parathion depending on flow rate, larval stage, and exposure time. It gave a 96-hour LC50 of 31 ug/l and chronic effect level of less than 3 ug/l, a lethal concentration in two weeks. The midge was the only species tested that is closely associated with sediment. The adsorption properties of parathion on the organic material used in the tests was probably the dominant influence on toxicity. Certainly the midge larvae could tolerate a relatively high single application of parathion if it were adsorbed rapidly by the substrate.

A few problems stand out in the methods for chronic toxicity testing of aquatic species. For seasonally applied pesticides, a one or two year chronic test of constant concentrations in water may not be a realistic approach. Only the shorter-lived invertebrates would under real conditions normally be exposed for chronic time periods. Bluegills are not practical species for presently conducted chronic bioassays unless a method is devised to ensure spawning during the first summer of laboratory exposure. For all fish bioassays, the egg handling, incubation and larvae rearing techniques recommended and used were cumbersome and required considerable labor. In many cases the harmful effects of handling outweighed the effect of the toxicant under invesigation. Among the invertebrates, Daphnia and Chironomus proved to be convenient test organisms. In long-term tests, Gammarus had inadequate survival using present culture techniques.

Finally, before accepting the results of such laboratory tests at face value, an additional understanding of pesticide effect in a natural system is needed. Mechanisms of direct uptake and trophic concentration should be considered, as well as microbial degradation, and adsorption on sediments. The effect on fish productivity and of reduction of invertebrates by pesticides should also be investigated.

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APPENDIX A

BIOASSAY DATA

TABLE A1. MEAN MONTHLY ANALYSES OF PARATHION, BLUEGILL CHRONIC TEST, ug/l

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Dec	nil	nil	4.54	4.87	2.44	2.60	1.23	1.00	0.50	0.57	(9.19)	(9.63)
Jan	0.01	0.01	4.40	4.10	2.12	2.37	1.06	1.04	0.45	0.40	(9.50)	(9.21)
Feb	0.01	0.01	3.80	3.70	2.25	2.11	0.94	0.91	0.36	0.37	0.14	0.14
Mar	0.02	0.02	4.77	4.74	2.44	2.27	1.30	1.31	0.58	0.61	0.17	0.18
Apr	0.03	0.03	2.93	2.50	1.29	1.24	0.70	0.65	0.32	0.34	0.02	0.10
May	0.01	0.01	2.38	2.51	1.21	1.12	0.64	0.63	0.32	0.34	0.11	0.13
June	0.01	0.02	3.72	3.52	1.83	1.93	1.09	1.04	0.39	0.48	0.19	0.20
July	0.02	0.02	2.08	1.99	0.99	1.03	0.57	0.64	0.25	0.26	0.12	0.12
Aug	0.01	0.01	3.03	3.07	1.55	1.54	0.75	0.75	0.44	0.46	0.19	0.23
Sept	0.06	0.06	2.27	2.27	1.19	1.19	0.50	0.50	0.18	0.18	0.12	0.12
Oct	0.07	0.07	4.96	4.96	1.74	1.74	0.57	0.57	0.22	0.22	0.13	0.13
Nov	0.04	0.03	2.39	2.58	1.24	1.15	0.42	0.35	0.17	0.06	0.09	0.05
Dec	0.02	0.02	2.44	2.44	1.87	1.87	0.80	0.80	0.27	0.27	0.06	0.06
Jan	0.01	0.01	3.80	3.80	1.58	1.58	1.45	1.45	0.13	0.13	0.08	0.08
Feb	0.04	0.04	1.81	1.81	1.26	1.26	0.47	0.47	0.15	0.15	0.08	0.08
Mar	0.10	0.10	0.97	0.97	0.37	0.37	0.19	0.19	0.11	0.11	0.17	0.17
Apr	0.17	0.17	3.29	3.29	1.86	1.86	1.18	1.18	0.31	0.31	0.40	0.40
May	0.12	0.12	2.00	2.00	0.93	0.93	0.92	0.92	0.22	0.22	0.14	0.14
June	0.21	0.21	2.88	2.88	1.62	1.62	1.33	1.33	0.39	0.39	0.29	0.29
July	0.04	0.04	4.72	4.72	2.07	2.07	1.98	1.98	0.49	0.49	0.26	0.26
Aug	0.19	0.20	3.77	2.78	2.16	1.97	1.33	1.33	0.56	0.56	0.38	0.38
Sept	0.12	0.12	2.83	2.81	1.20	1.18	1.39	1.39	0.75	0.79	0.17	0.16
Oct	0.03	0.03	5.04	5.03	2.29	2.33	2.53	2.55	0.44	0.44	0.32	0.31
Mean*	0.06	0.06	3.23	3.14	1.53	1.59	1.00	1.00	0.34	0.34	0.16	0.17
SD*	0.09	0.09	1.88	1.83	0.98	1.03	0.74	0.74	0.26	0.26	0.14	0.14
High	0.39	0.39	10.59	10.59	4.23	3.95	3.01	3.01	1.03	1.08	1.10	1.10
Low	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

*Based on weekly samples.
nd: not detectable.

TABLE A2. ROUTINE WATER QUALITY, BLUEGILL CHRONIC TEST

	N	Mean	Range
D.O., mg/l	1008	6.1	3.8-7.3
pH	252	7.80	7.62-8.20
Alkalinity, mg/l	252	157	142-172
Acidity, mg/l	252	6.4	0.3-13.1
Hardness, mg/l	252	265	158-309

TABLE A3. MEAN MONTHLY TEMPERATURES, BLUEGILL CHRONIC TEST

Month	Deg C	Month	Deg C	Month	Deg C
Jan	23.0	Sept	26.4	May	25.0
Feb	24.2	Oct	27.1	June	25.1
Mar	25.0	Nov	26.0	July	27.2
Apr	24.7	Dec	26.9	Aug	27.2
May	25.6	Jan	24.7	Sept	26.6
June	26.1	Feb	24.4	Oct	
July	26.2	Mar	24.4	Nov	
Aug	26.6	Apr	25.7		

TABLE A4. BLUEGILL SURVIVAL, CHRONIC TEST

		Tank											
Date		0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
1971	Dec 1	20	20	20	20	20	20	20	20	20	20	..	..
1972	Mar 1	20	20	17	13	20	17	17	17	20	20	49*	11*
	May 1	19	20	17	12	20	17	17	17	19	20	34	10
	Oct 1	19	19	17	13	20	17	17	15	18	17	25	10
1973	Mar 1	18	19	17	13	20	17	17	15	14	14	12	10
	May 1	13	17	17	13	20	17	16	15	9	13	12	10
	Jul 1§	10	15	17	13	20	17	16	15	9	13	10	9
	Nov 1	9	9	8	8	11	9	9	9	8	9	7	7

*Eleven of same stock started in Tank 5B Feb 25, 1972; 49 from new stock started in Tank 5A Feb 25, 1972.
§Later thinned to 3 males, 7 females per tank.

TABLE A5. BRAIN ACETYLCHOLINESTERASE, BLUEGILLL, 18 MONTH EXPOSURE, umol/min/g wet weight

	Tank					
	1	2	3	4	5	0
	1.26	3.14	3.03	6.34	7.35	12.97
	1.36	3.47	2.92	7.40	7.43	7.16
	1.28	4.07	3.22	5.88		5.07
	1.74	4.22	3.19	6.74		5.35
	1.23	4.98	2.10			6.04
	1.66	4.35	2.15			
	2.16	4.23	2.94			
	0.76	3.26	3.78			
	1.22	4.14	3.17			
	1.03	2.32	2.51			
			2.46			
Mean	1.37	3.35	2.86	6.59	7.39	7.32
Percent of control	18.7	45.8	39.1	90.0	101	100

TABLE A6. BRAIN ACETYLCHOLINESTERASE, BLUEGILL 23 MONTH EXPOSURE, umol/min/g wet weight

	Tank					
	1	2	3	4	5	0
	1.09	2.07	1.21	3.64	8.15	4.53
	1.15	1.41	1.75	5.37	5.85	9.76
	1.95	2.53	1.38	3.07	7.46	12.20
		1.92				7.49
Mean	1.40	1.98	1.45	4.03	7.15	8.50
Percent of control	16.5	23.3	17.0	47.4	94.1	100

TABLE A7. BROOK TROUT ACUTE TEST

a. Number surviving per tank

	Tank											
Hour	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
0	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	0	0	10	10	10	10	10	10	10	10
48	10	10	0	0	10	10	9	10	10	10	10	9
96	10	9	0	0	5	4	8	9	10	9	10	9

b. Analyzed parathion concentration, mg/l

0	nd	nd	3.30	3.06	1.59	1.59	0.88	0.91	0.43	0.47	0.21	0.25
48	nd	nd	4.84	4.60	1.96	2.11	1.06	1.00	0.51	0.52	0.24	0.28
96	nd	nd	4.41	3.79	1.94	1.96	1.04	0.86	0.43	0.42	0.25	0.26
Mean			4.18	3.82	1.83	1.89	0.99	0.92	0.46	0.47	0.23	0.26
SD			0.79	0.77	0.21	0.27	0.10	0.07	0.05	0.05	0.02	0.02

nd: not detectable.

c. Mean weight of fish, gm

81.1	83.8	86.0	78.2	83.6	89.6	73.1	82.4	68.4	87.7	74.3	82.9

TABLE A8. ROUTINE WATER QUALITY, BROOK TROUT CHRONIC

	N	Mean	Range
D.O., mg/l	504	8.2	6.7-10.5
pH	280	7.81	7.47-8.06
Alkalinity, mg/l	312	154	150-183
Acidity, mg/l	284	6.0	4.0-10.0
Hardness, mg/l	284	295	256-342

TABLE A9. MEAN MONTHLY TEMPERATURES, BROOK TROUT CHRONIC

Month	Deg C±2	Month	Deg C±2	Month	Deg C±2
May	14.0	Sept	16.7	Jan	13.1
June	13.8	Oct	15.0	Feb	13.6
July	14.2	Nov	13.4	Mar	14.2
Aug	15.8	Dec	13.3		

TABLE A10. MEAN MONTHLY ANALYSIS OF PARATHION IN WATER, BROOK TROUT CHRONIC TEST, ug/l

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
May	0.07	0.07	9.50	8.40	3.41	3.27	1.84	1.83	0.86	0.94	0.40	0.46
June	0.04	0.05	6.12	6.40	2.70	2.57	1.59	1.53	0.63	0.63	0.46	0.44
July	0.04	0.03	9.49	9.52	4.43	4.50	2.94	2.84	0.76	1.07	0.59	0.62
Aug	0.02	0.01	11.86	11.70	5.36	5.30	3.84	3.84	0.91	0.90	0.36	0.42
Sept	0.02	0.02	1.32	1.32	0.80	0.80	0.59	0.59	0.31	0.31	0.11	0.11
Oct	0.16	0.16	7.19	7.19	3.77	3.77	2.56	2.56	1.11	1.11	0.29	0.29
Nov	0.02	0.02	3.68	4.84	2.16	2.54	0.94	0.89	0.83	0.83	0.21	0.21
Dec	0.01	0.01	3.77	3.77	2.15	2.15	0.72	0.72	0.42	0.42	0.11	0.11
Jan	0.01	0.01	6.62	6.62	3.06	3.06	2.13	2.13	1.12	1.12	0.13	0.13
Feb	0.05	0.07	9.30	9.73	4.96	4.53	3.25	3.26	1.49	1.47	0.60	0.65
Mar	0.01	0.01	12.34	12.34	6.03	6.02	4.43	4.43	1.92	1.92	1.07	1.07
Mean	0.05	0.05	7.12	7.17	3.38	3.35	2.12	2.10	0.88	0.92	0.34	0.36
N	45	45	45	45	45	45	45	45	45	45	45	45
SD	0.11	0.11	4.34	4.17	1.99	1.98	1.44	1.41	0.62	0.60	0.31	0.32
High	0.23	0.23	16.90	14.00	6.74	6.82	5.01	5.01	2.30	2.30	1.06	1.14
Low	nil	nil	0.28	0.28	0.62	0.62	0.06	0.06	0.19	0.19	0.02	0.01

TABLE A11. BROOK TROUT SURVIVAL IN CHRONIC EXPOSURE

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
May 1	14	14	14	14	14	14	14	14	14	14	14	14
Jun 1	14	6	10	7	14	13	0*	12	13	13	14	14
Jul 1	14	5	8	6	14	1	0	12	13	13	14	13
Jul 19	14	5	8	6	15**	9**	12**	12	13	13	14	13
Aug 1	14	4	8	6	15	7	9	12	7	12	14	13
Sep 1	14	4	8	6	15	7	9	12	7	12	14	13
Oct 1	14	4	8	6	12	7	8	12	6	12	14	13
Oct25§	14	4	8	6	12	6	7	12	6	12	14	13
Nov 1	6	6	6	6	6	6	6	6	6	6	6	6
Dec 1	6	6	6	6	6	6	6	4	5	6	6	6
Jan 1	6	6	6	6	6	6	6	4	4	6	6	6
Feb 1	6	6	5	6	5	6	6	4	4	5	6	6
Feb 6	6	6	5	5	5	6	6	4	4	5	5	6

*Complete mortality from low flow and dissolved oxygen, May 27.
**One remaining fish from 2B moved to 2A on July 19. Nine new fish added to 2B, twelve added to 3A.
§Thinning. All tanks reduced to 2 males, 4 females. All fish from 2B and 3A removed. Six fish transferred from each corresponding duplicate.

TABLE A12. BROOK TROUT WEIGHTS, CHRONIC EXPOSURE

Tank	May 1			August 25			February		
	Mean weight, gm	No.	S.D.	Mean weight, gm	No.	S.D.	Mean weight, gm	No.	S.D.
0A	63.9	14	12.8	182.3	14	32.8	331.7	6	80.7
0B	62.8	14	11.1	201.9	4	64.8	439.7	6	78.3
1A	60.6	14	8.8	179.7	8	42.5	347.6	5	72.8
1B	62.6	14	13.6	204.2	6	25.2	436.2	5	78.6
2A	60.6	14	6.9	163.8	15	17.4	370.2	5	119.9
2B	67.6	14	9.5	...	..	...	290.5	6	47.7
3A	60.0	14	8.4	...	..	...	394.5	6	100.6
3B	68.2	14	8.7	198.2	12	19.2	320.8	4	17.3
4A	67.0	14	10.5	209.0	7	28.6	358.8	4	149.7
4B	59.3	14	8.1	184.9	12	21.5	337.8	5	63.7
5A	59.9	14	8.2	172.4	14	20.4	351.2	5	45.9
5B	63.0	14	10.0	183.3	13	31.9	416.2	6	74.6

TABLE A13. BROOK TROUT GONADOSOMATIC INDEX, SIX MONTH EXPOSURE, PERCENT

	Tank							
	0A	1A	2B	3A	4A	4B	5A	5B
Female	0.21	0.28	1.27	3.87	0.30		0.33	0.31
	0.34	0.24		3.11	0.36		9.75	0.34
				4.25			7.10	5.01
				5.65				2.74
Male	0.78		1.08	0.96	1.32	1.53	2.26	1.74
	0.53		1.90	2.38	1.19	1.29	7.16	0.39
	0.37		1.12	1.24			0.29	2.18
	2.88		1.88				1.73	
			1.25				2.51	

TABLE A14. BROOK TROUT GONADOSOMATIC INDEX, NINE MONTH EXPOSURE, PERCENT

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Female	0.77	0.65	0.05	0.68	0.02	0.41	0.48	0.70	0.39	0.05	0.51	0.78
	0.51	0.48	0.58	0.62	0.04	nil	0.14	nil	0.49	nil	0.47	0.10
	1.26	nil	1.49	nil	0.67	1.00	0.46		0.53	0.70	0.33	0.50
	0.50	0.28	0.35	nil	0.43	0.63	0.58			0.57	0.50	0.42
					0.40	0.41						
Male	0.26	3.39	1.68	0.45		0.26	1.06	0.38	0.18	2.36	0.14	0.36
	0.34	0.56						0.70				0.08

TABLE A15. SUMMARY OF RESIDUE RESULTS FOR BLOOD AND MUSCLE OF TROUT EXPOSED TO PARATHION FOR SIX AND NINE MONTHS

Tank no.	Avg water conc, ppb	No. of fish	Months exposure	Residue, ppb		Conc ratio, B/T
				Blood (B)	Tissue(T)	
21	7.1	2	6	1302	3842	0.34
		5	9	805	1260	0.64
22	3.4	3	6	788	1884	0.42
		5	9	361	435	0.83
23	2.1	5	6	603	1142	0.53
		5	9	256	306	0.84
24	0.90	3	6	126	419	0.30
		5	9	107	59	1.81
25	0.35	4	6	68	171	0.40
		5	9	43	52	0.83

TABLE A16. ROUTINE WATER QUALITY, FATHEAD MINNOW CHRONIC TEST

	N	Mean	Range
D.O.,mg/l	384	7.1	8.1-6.3
pH	90	7.94	8.05-7.78
Alkalinity, mg/l	90	153	168-135
Acidity, mg/l	90	2.5	9.1-10.5
Hardness, mg/l	90	238	261-194

TABLE A17. MEAN MONTHLY WATER TEMPERATURE, FATHEAD MINNOW CHRONIC TEST

Month	Deg C	Month	Deg C	Month	Deg C
Apr	24.8	July	23.8	Oct	24.3
May	23.9	Aug	23.2	Nov	23.5
June	23.5	Sept	24.2	Dec	24.3

TABLE A18. MEAN MONTHLY ANALYSIS OF PARATHION IN WATER, FATHEAD MINNOW CHRONIC TEST, ug/l

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Apr	0.30	0.24	50.76	53.47	20.17	24.30	23.13	24.00	12.90	13.37	4.31	4.75
May	0.04	0.04	42.06	42.06	19.87	19.87	13.10	13.10	6.80	6.80	4.88	4.88
June	0.12	0.12	59.39	59.39	26.56	26.56	17.36	17.36	11.07	11.07	4.82	4.82
July	0.07	0.06	53.88	46.33	20.86	21.41	15.11	10.82	8.99	8.26	3.53	3.60
Aug	0.11	0.06	41.17	42.94	18.08	19.96	13.28	14.57	8.77	7.78	4.98	5.56
Sept	0.34	0.30	48.62	48.68	20.06	17.75	14.48	13.00	8.39	7.48	4.43	4.05
Oct	0.06	0.06	40.25	39.06	18.82	18.31	10.85	10.58	5.37	5.39	3.39	3.41
Nov	0.02	0.02	58.28	58.12	28.52	27.99	16.13	15.31	8.44	6.83	2.64	3.85
Dec	0.02	0.04	55.56	53.25	27.07	23.50	15.00	12.21	7.98	8.18	4.25	3.97
Mean	0.14	0.12	49.40	48.53	21.51	21.94	15.61	15.36	8.95	9.02	4.46	4.36
SD	0.14	0.18	18.08	15.06	8.16	7.18	7.55	7.40	4.14	2.87	1.55	1.18
High	0.82	0.82	104.20	104.20	44.86	44.86	38.70	38.70	17.90	17.90	7.30	7.30
Low	nil	nil	8.61	16.81	3.19	6.70	2.06	3.34	2.81	2.24	1.13	1.60

TABLE A19. GROWTH AND SURVIVAL OF FATHEAD MINNOWS IN PARATHION

	30 days		60 days		
Tank	Number present	Mean length, mm	Number present	Mean length, mm	Mean growth, mm
0A	20	14.7	19	22.6	7.9
0B	33	14.6	28	22.3	7.7
1A	27	13.8	23	20.2	6.4
1B	28	15.2	27	23.0	7.8
2A	24	14.9	24	19.7	4.8
2B	28	14.1	27	19.6	5.5
3A	25	15.5	23	23.2	7.7
3B	28	13.8	26	19.3	5.5
4A	26	16.0	23	22.4	6.4
4B	31	14.5	29	20.4	5.9
5A	23	16.4	23	23.3	6.9
5B	26	14.8	22	23.8	9.0

Number at start: 35 per tank.

TABLE A20. DAPHNIA MAGNA ACUTE TEST, FLOW-THROUGH, 18°C

a. Number surviving

	Tank									
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	7	7	10	7	7	10	9	9
48 hr	10	10	0	0	8	4	7	6	9	9
72 hr	10	10	0	0	8	3	7	5	9	9
96 hr	10	10	0	0	5	3	7	4	9	9

b. Parathion, ug/l

0.11	0.10	1.56	1.55	0.85	0.86	0.45	0.44	0.23	0.24

c. LC50, ug/l

24 hour	2.70 (5.61 - 1.30)
48 hour	1.00 (1.73 - 0.58)
96 hour	0.62 (0.90 - 0.43)

TABLE A21. DAPHNIA MAGNA CHRONIC TEST

a. Number surviving

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
1 wk	10A 89Y	9A 34Y	0	0	0	0	10	9	10	10	10	10
2 wk	8A 476Y	6A 589Y	0	0	0	0	8A 271Y	8A 233Y	10A 387Y	9A 219Y	10A 375Y	10A 416Y
3 wk	7A 205Y	3A 51Y	0	0	0	0	0A 103Y	0A 176Y	9A 268Y	8A 280Y	10A 114Y	9A 367Y
Total young	770	674	...	...	...	...	374	409	655	499	489	783

A: adult, Y: young.

b. Parathion analysis, ug/l

Start	0.01	0.02	0.80	0.79	0.37	0.38	0.28	0.27	0.16	0.16	0.09	0.09
1 wk	0.02	0.02	0.72	0.70	0.31	0.30	0.20	0.20	0.10	0.10	0.08	0.08
2 wk	0.02	0.02	0.85	0.85	0.48	0.47	0.23	0.23	0.10	0.10	0.08	0.07

c. LC50, ug/l

1 week	0.28 (0.29 - 0.26)
2 week	0.25 (0.22 - 0.28)
3 week	0.14 (0.16 - 0.13)

RI50: 0.24 (0.32 - 0.17)
MATC: 0.08 ug/l

TABLE A22. GAMMARUS ACUTE TEST

a. Number surviving

	Tank									
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	6	5	7	6	10	10	10	10
48 hr	10	10	0	0	0	0	9	10	10	10
72 hr	10	10	0	0	0	0	8	7	9	10
96 hr	10	10	0	0	0	0	5	5	8	10

b. LC50, ug/l

24 hour	2.1 (3.8 - 1.2)*
48 hour	0.62
96 hour	0.43 (0.65 - 0.29)

*For juveniles at 20°C.

TABLE A23. GAMMARUS SUBCHRONIC TEST, 20°C, NUMBER SURVIVING.

	Tank									
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B
Start	20	20	20	20	20	20	20	20	20	20
8 days	20	20	0	14	16	17	16	15	19	18
23 days	16	19	0	0	1	0	4	7	15	13
30 days	15	15	0	0	0	0	0	1	6	7
36 days	13	14A 8Y	0	0	0	0	0	1	6A 5Y	7A 4A
43 days	13	14A	0	0	0	0	0	1	3A	7A
Mean parathion conc, ug/l	0.03	0.02	1.64	1.65	0.24	0.24	0.15	0.15	0.04	0.05

A: adults, Y: young.

TABLE A24. CHIRONOMUS TENTANS ACUTE TEST

a. Number surviving

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	10	10	10	10	10	10	10	10	10	10
48 hr	10	10	5	8	6	9	10	10	10	10	10	10
72 hr	10	9	2	2	5	7	9	9	10	8	10	10
96 hr	10	9	0	0	5	4	9	8	9	8	10	10

b. Analyzed parathion, ug/l

Start	0.14	0.14	66.3	66.3	33.6	33.6	17.6	17.6	5.7	5.7	5.8	5.8
72 hr	0.09	0.09	48.6	48.6	33.0	33.0	17.2	17.2	6.2	6.2	4.0	4.0

c. LC50, ug/l

48 hour	135 (703 - 26)*
96 hour	31.0 (43.4 - 22.1)

*For 4th instar larvae at 21°C.

TABLE A25. CHIRONOMUS TENTANS SUBCHRONIC TEST

a. Number surviving

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
4 days	10	10	6	8	10	8	10	9	9	10	9	9
7 days	10		0	4	8	6	7	9	8	6	8	7
14 days	10E	10E	0	0	0	0	3	4	3	1	3	4

b. Analyzed parathion, ug/l

	0.3	0.3	52.9	52.9	23.9	23.6	11.0	11.0	5.0	5.1	3.1	3.1

c. LC50, ug/l

14 days	2.3 (3.4 - 1.6)

APPENDIX B

BROWN TROUT ACUTE TEST

Young brown trout, Salmo trutta Linnaeus, 16 to 19 cm total length, were acutely exposed to parathion in an LC50 test and also in a residue uptake test. The acute LC50 test method was identical to that of the brook trout. The LC50 results for 24 and 96 hours (Table B1) show essential agreement with those for brook trout. Symptoms of parathion poisoning were also similar.

TABLE B1. BROWN TROUT ACUTE TEST

a. Number surviving

	Tank											
	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
24 hr	9	10	6	0	10	10	10	10	10	10	10	10
48 hr	9	10	0	0	9	10	10	10	10	10	10	10
96 hr	9	10	0	0	5	1	10	10	10	10	10	10
144 hr	9	10	0	0	0	0	9	6	10	1	10	10

b. Analyzed parathion concentration, ug/l

Pooled daily	nd	nd	2.40	2.40	1.63	1.63	1.04	1.04	0.68	0.68	0.47	0.47

c. LC50, ug/l

Hour	LC50	Upper CL	Lower CL
24	2.15	2.34	1.97
48	1.93	2.01	1.85
96	1.51	1.65	1.38
144	1.13	1.03	1.24

In a separate test, brown trout of the same stock were exposed to the two highest parathion levels which had caused no acute mortalities, 0.68 and 0.47 mg/l. A total of 45 fish were used in each of the two tanks. During the parathion exposure period, three fish were removed from each tank at selected intervals up to 64 hours. At 65 hours, the remaining fish were transferred to two duplicate tanks with uncontaminated flowing water. Again three fish per tank were removed at time intervals until all fish were gone at 79 hours. Muscle samples dissected from the dorsal fin region were preserved at -18°C and later analyzed for parathion residues. The residue values shown in Table B2 are each the mean of true duplicates. Precision for the clean-up and chromatographic method was $\pm$5 percent.

A simple model was applied to the data from the 0.47 mg/l exposure and plotted (Figure B1). Assuming first order kinetics, the change in fish residue, C, can be expressed as a function of C_w, the toxicant concentration in water:

$$dc/dt = k_1 C_w - k_2 C$$

$$C = K_1 C_w / k_2 + [(C_o - k_1 C_w)/k_2] e^{-k_2 t}$$

where k_1 is the rate constant for uptake from water, k_2 is the rate of elimination, metabolism, or other removal, and C_o is the initial fish residue. The washout data were fitted to a least-square line of log C vs. time, giving $K_2 = 0.002$/hr. Substituting for k_2 and C at 64 hours, the value of K_1 is about 1.0/hr. The calculated stady state level of parathion in the muscle is 250 mg/kg, reached in about 90 days, assuming no mortality or change in mechanism. The concentration factor of 500 then agrees very well with the concentration factors for chronically exposed brook trout muscle. Although the data are not as precise as one would like, they show that a simple model can be used to describe parathion uptake in fish. The rate constants would, of course, vary with the temperature, fish species, and, perhaps, order of magnitude of the dosage.

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TABLE B2. PARATHION RESIDUES IN BROWN TROUT MUSCLE

Parathion in water, mg/l		0.47	0.68
Uptake residues, mg/kg	2 hr	4.10	5.48
		4.53	10.61
	4 hr	4.87	7.48
		6.48	
	8 hr	3.11	10.91
		4.63	12.45
	16 hr	9.08	7.98
		11.95	16.66
	32 hr	21.78	15.79
		23.64	43.34
	64 hr	22.88	46.49
		34.80	57.99
Washout residues, mg/kg	2 hr		64.05
			72.85
	3 hr	22.74	
		42.21	
	4 hr		29.44
			66.92
			33.24
	4.5 hr	8.27	
		27.66	
	16 hr	22.38	41.78
		33.94	44.79
	35 hr	7.90	22.53
		26.23	42.50
	64 hr	23.14	21.29
		25.60	55.74
	77 hr		28.13
			22.76
			44.52
	78 hr	13.89	18.96
		27.38	48.35
	79 hr	12.59	36.20
		32.77	47.96

True duplicates analyzed at each time period. Precision ±5%.

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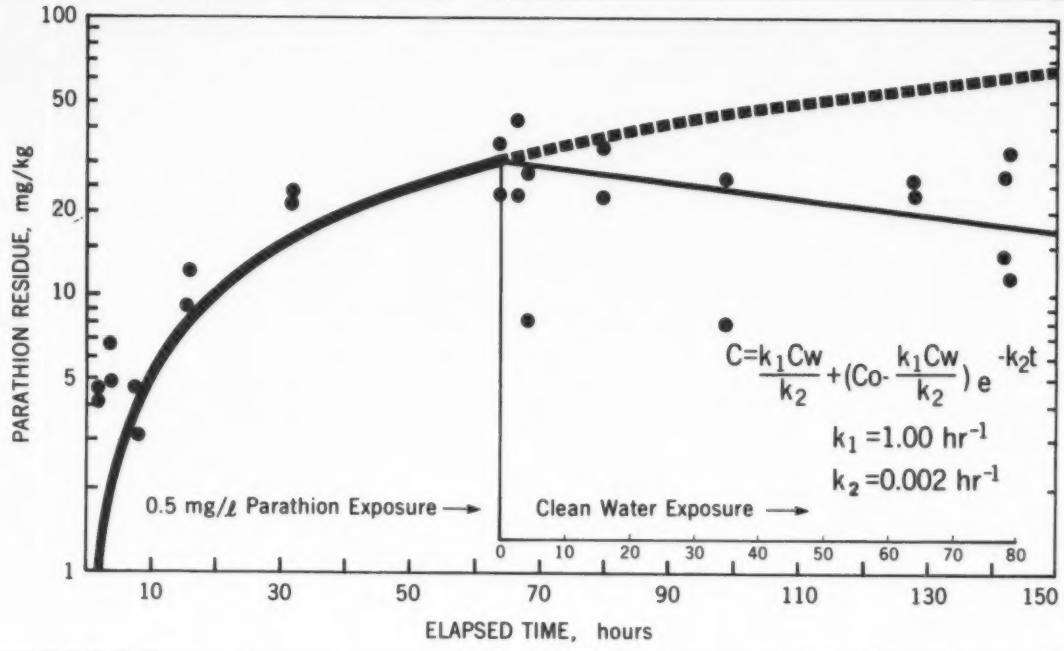

Figure B1. Parathion uptake and washout in brown trout muscle, 12°C.

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APPENDIX C

CHLORINATED HYDROCARBON PESTICIDES IN FISH FOOD

Samples of fish food used for chronic tests were analyzed for chlorinated hydrocarbon pesticides. Initial attempts to attain effective clean-up of these food samples using standard Florisil chromatography failed. The silicic acid chromatography procedure reported by Kadoum (46) was subsequently used. Food samples were extracted with hexane in the presence of Na_2SO_4. Concentrated extracts were applied to columns of silicic acid (Mallinckrodt 100 mesh, chromatography grade) and eluted with hexane and 2%, 10%, 40% and 70% benzene in hexane and benzene. Fractions were pooled, evaporated and rechromatographed a second time with collection of individual fractions: hexane, 2% benzene, 10% benzene, 40% benzene, 70% benzene, and benzene. These were analyzed by GC on 3% OV - 17 on Chromosorb W at 195°C using electron capture detection. Standard solutions of 13 chlorinated hydrocarbon pesticides were chromatographed to provide retention times (RT) for identification purposes and peak areas (as counts) for quantification. The conditions of elution from the silicic acid and the GC RT's were used for tentative identifications. Good matches, however, were not obtained for heptachlor epoxide and lindane and nearest peaks were used; all peaks eluted in the DDT and DDT degradation product area were summed and calculated as DDT+DDD+DDE. These, therefore, are maximum values. From the blood samples, resolvable peaks distinct from the interferences could not be obtained in the retention time regions of dieldrin or endrin in the 70% benzene fraction. The presence at low level (ppb) or absence of these pesticides could not be verified. The overall results are summarized in Table C1. In general it was concluded that chlorinated hydrocarbon pesticide levels were low in each food source used and should not have had a significant affect on the tests.

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TABLE C1. PESTICIDE RESIDUES IN FISH FOOD, ug/kg

Food sample	Aldrin	Heptachlor	DDE DDD DDT	Heptachlor Epoxide*	Lindane**	Dieldrin Endrin§
Starter mash	<1	<0.1	<0.5	nil	<0.3	ND
No. 3 pellet	nil	<0.2	<0.5	nil	<0.3	ND
No. 6 pellet	nil	<0.1	<0.5	nil	<0.6	ND
No. 5 pellet	§§	<0.1	<0.5	<1	<1	ND

*No true match of retention time (RT) with standard; nearest GLC peak taken as heptachlor epoxide for calculation of no. 5 sample.

**No true match of RT with standard; nearest peak taken as lindane for calculation. RT was 256 sec. vs. 249 sec. for lindane standards. Peak may be a phthalate ester.

§These pesticides could not be detected (ND) unless present at very high levels because of broad peaks of relatively high level interferences present in the 70 percent benzene fraction.

§§A GLC peak near the RT of aldrin, but probably not aldrin, gave a value of 10-15 ppb calculated on the basis of aldrin.

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